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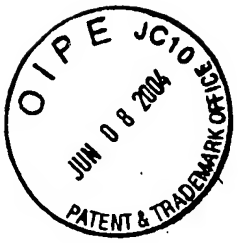


DAG
IFW

SALIWANCHIK, LLOYD & SALIWANCHIK
A Professional Association

2421 N.W. 41st Street
Suite A-1
Gainesville, Florida 32606-6669
Telephone 352-375-8100
Facsimile 352-372-5800

Roman Saliwanchik
(1926 - 1999)

June 7, 2004

VIA FACSIMILE

Ms. Pat Paxton
Landon Stark Cantwell & Paxton
2011 Crystal Drive
Suite 210
Arlington, VA 22202-3709

Confirmation by Courier

Re: U.S. Patent Application Docket No. UF-375
NOVEL MELANOCORTIN RECEPTOR TEMPLATES, PEPTIDES, AND USE THEREOF
(Carrie Haskel-Luevano)
Serial No. 10/602,394; filed June 23, 2003
Your Ref. No. UF#-11169

Dear Pat:

Enclosed with the confirmation copy of this letter is a package relating to the above-referenced matter that we would like hand-delivered to Examiner Paul Shanoski at the U.S. Patent Office. Mr. Shanoski is located in Office of Petitions, located at Crystal Plaza Two, Lobby, Room IB03, Arlington, VA 22202.

Also, please have the attached postcard date stamped by the U.S. Patent Office and then returned to us.

Thank you for your assistance in this matter.

Sincerely,

Dave

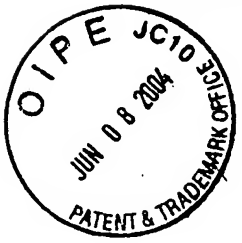
David R. Saliwanchik

DRS/la

Enclosures: as stated above

H:\DOC\DRS\PAXTON-UF-375.DOC/DNB/la

HAND-DELIVERED



RENEWED PETITION
UNDER 37 C.F.R.§1.182
Patent Application
Docket No. UF-375
Serial No. 10/602,394
Conf. No. 1696

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Carrie Haskell-Luevano
Serial No.	:	10/602,394
Filed	:	June 23, 2003
Art Unit	:	1646
For	:	Novel Melanocortin Receptor Templates, Peptides, and Use Thereof

ATTN: Paul Shanoski, Esq.

Office of Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

RENEWED PETITION UNDER 37 C.F.R.§1.182

Sir:

This Renewed Petition is in response to the Decision of Petition dated April 23, 2004 received from the Office of Petitions in the above-referenced patent application.

The applicant wishes to thank Examiner Shanoski for his careful review of the Petition and his direction to correct the deficiencies as noted.

As instructed in the Decision on Petition, this Renewed Petition only addresses the deficiencies noted in the Decision of Petition.

Specifically, the accompanying Declaration of Facts, and its attachments more clearly and explicitly set forth the details upon which the applicant bases her belief that a Notice to File Missing Parts was never received by the applicant's representative for this case.

For example in the attached Declaration of Facts, the undersigned states that the missing Notice did not appear on the firm's list of incoming mail or on the firm's Docket Report. Also, upon inspection of the relevant case folder, it was determined that the missing Notice was not in the file. A copy of the Docket Report and the file jacket have been provided.

As a point of clarification, in the applicant's original Petition under 37 CFR §1.182 the Petitioner requested that the Declaration be considered "timely filed." What the Petitioner meant by this was that no extension of time should be charged for responding to the (missing) Notice to File Missing Parts. The applicant apologizes for any confusion regarding this issue.

In view of this Renewed Petition and the accompanying Declaration of Facts, the applicant respectfully requests favorable consideration of this Renewed Petition.

The Commissioner is hereby authorized to charge any additional fees that may be required to Deposit Account No. 19-0065.

Respectfully submitted,

David Saliwanchik

David R. Saliwanchik
Patent Attorney
Registration No. 31,794
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: 2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669

June 7, 2004
Date

DRS/la

Attachments: Copy of File Jacket
Declaration of Facts
Docket Report

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	:	1646
Applicant(s)	:	Carrie Haskell-Luevano
Serial No.	:	10/602,394
Conf. No.	:	1696
Filed	:	June 23, 2003
For	:	Novel Melanocortin Receptor Templates, Peptides, and Use Thereof

Office of Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ATTN: Paul Shanoski, Esq.

DECLARATION OF FACTS BY DAVID R. SALIWANCHIK

IN SUPPORT OF RENEWED PETITION UNDER 37 CFR §1.182

Sir:

I, DAVID R. SALIWANCHIK, hereby declare:

THAT, I am a partner in the law firm of Saliwanchik, Lloyd & Saliwanchik (SLS);

THAT, I have been with SLS for 17 years and I am very familiar with the firm's procedures for receiving mail and docketing items received in the mail;

THAT, the attorneys of SLS are the attorneys of record on the above-referenced patent application; and

Being thus duly qualified, do further declare as follows:

THAT, each piece of incoming mail from the U.S. Patent Office received by SLS is recorded by our docketing department, and entered into our computerized docket system;

THAT, I have reviewed a confidential list of all of the incoming correspondence received by our firm for September 2003 through January 15, 2004, and that this list of incoming correspondence does not indicate receipt of any correspondence from September 8, 2003 to January 15, 2004 from the U.S. Patent Office relating to U.S. Patent Application No. 10/602,394;

THAT, each day our docket department generates a list (Docket Report) of the items due in the Patent Office that day. I have reviewed a copy of our Docket Report for November 11, 2003 (this is the day a Response to the missing Notice would have been due) and the Docket Report does not show any response due for a Notice to File Missing Parts for U.S. Patent Application No. 10/602,394. A copy of that Docket Report is attached hereto.

THAT, I have reviewed our case folder for U.S. Serial No. 10/602,394, where the Notice to File Missing Parts would be filed and kept after docketing, and I have not found the Notice to File Missing Parts. A copy of the file wrapper is enclosed.

THAT, based on the facts set forth above, I conclude that, prior to January 16, 2004, SLS did not receive a Notice to File Missing Parts for U.S. Patent Application No. 10/602,394.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By: David Saliwanchik
David R. Saliwanchik

June 7, 2004
Date

Docket Report

Events Copy of Simple Listing

[Uncompleted Docket and [Event Class contains 'U.S.'] and [no reminders] and [Event Date >= Ask User('Beginning Date') and Event Date <= Ask User ('Ending Date') and has(Rules Set)]

Matter ID	Responsible	Notes
Items Due November 11, 2003 (Tuesday)		
UF-251	JMS	Patent issued (for patents filed on or after 06/08/1995)
UF-292	GPL	Patent issued (for patents filed on or after 06/08/1995)
BRR-MISC	DRS,MHE	Opinions Method and System for Assessing Satisfaction 10/07/2003 - Transmitted instructions to Patent Providers to conduct search. 10/29/2003 - Rec'd search from Patent Providers.
BTY-100C1	JEK	Issue Fee Due: 09/17/2003 Issue Fee Paid: 09/16/2003 Drawings Accepted? YES File a Continuation/Divisional? BTY-100C2 ; Filed: 11/10/2003 (awaiting paperwork) Check: Issue Notification Received? Yes (6,644,302 11/11/2003) NOA to Bartley 06/25/2003; requested confirmation to file cip/cont
BTY-100C1	JEK	Patent issued (for patents filed on or after 06/08/1995) ADD MATTER ID TO SLS-MISC ISSUED CASES LIST AND UPDATE NUMBER
BTY-100C2	JEK	Target Filing Date (BTY-100C1 will issue on 11/11/2003) DATE instructions received: 03/04/2003 11/10/2003 - Per JEK filed, awaiting paperwork
Items Due November 12, 2003 (Wednesday)		
G-021US03DIV	FCE	Check: Notice to File Missing Parts of Nonprovisional Application received? Date of Notice:
G-021US03DIV	FCE	Check: first filing receipt received? Date Application Filed: 07/30/2003
G-076US11CON	FCE	Miscellaneous Formalities (no specific deadlines) Executed Dec/POA Original Date: 02/28/2003

UNITED STATES PATENT AND TRADEMARK OFFICE

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UNITED STATES PATENT AND TRADEMARK OFFICE
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450
www.uspto.gov

Paper No. None

SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
2421 N.W. 41ST STREET
SUITE A-1
GAINESVILLE FL 32606-6669

In re Application of
Carrie Haskell-Luevano
Application No. 10/602,394
Filed: June 23, 2003
Attorney Docket No. UF-375
Title: NOVEL MELANOCORTIN RECEPTOR TEMPLATES, PEPTIDES, AND USE THEREOF

DECISION ON PETITION
UNDER 37 C.F.R. §1.182

BACKGROUND

This is a decision on the petition under 37 C.F.R. §1.182, filed February 13, 2004, requesting that the executed declaration associated with the above-identified application be accepted as timely filed, and that the petition fee be waived.

The above-identified application was deposited on June 23, 2003, listing Carrie Haskell-Luevano as the sole inventor, with a declaration which was not executed. On September 8, 2003, applicant was mailed a "Notice to File Missing Parts of Nonprovisional Application - Filing Date Granted" (notice), requiring an executed oath or declaration in compliance with §1.63 and a $65 surcharge for its late filing. This Notice set a two-month period for reply.

With the instant petition, Petitioner has submitted a declaration which has been executed by the sole inventor along with the surcharge associated with the late submission of the same, a declaration of facts, an assertion that the notice was not received, and a request to have the petition fee waived and the declaration considered timely filed.

THE RELEVANT SECTION OF THE MPEP

MPEP 711.03(c) states, in part:

PETITION TO WITHDRAW HOLDING OF ABANDONMENT BASED ON FAILURE TO RECEIVE OFFICE ACTION

In Delgar v. Schulyer, 172 USPQ 513 (D.D.C. 1971), the court decided that the Office should mail a new Notice of Allowance in view of the evidence presented in support of the contention that the applicant's representative did not receive the original Notice of Allowance. Under the reasoning of Delgar, an allegation that an Office action was never received may be considered in a petition to withdraw the holding of abandonment. If adequately supported, the Office may grant the petition to withdraw the holding of abandonment and remail the Office action. That is, the reasoning of Delgar is applicable regardless of whether an application is held abandoned for failure to timely pay the issue fee (35 U.S.C. 151) or for failure to prosecute (35 U.S.C. 133).

To minimize costs and burdens to practitioners and the Office, the Office has modified the showing required to establish nonreceipt of an Office action. The showing required to establish nonreceipt of an Office communication must include a statement from the practitioner stating that the Office communication was not received by the practitioner and attesting to the fact that a search of the file jacket and docket records indicates that the Office communication was not received. A copy of the docket record where the nonreceived Office communication would have been entered had it been received and docketed must be attached to and referenced in practitioner's statement. For example, if a three month period for reply was set in the nonreceived Office action, a copy of the docket report showing all replies docketed for a date three months from the mail date of the nonreceived Office action must be submitted as documentary proof of nonreceipt of the Office action. See Notice entitled "Withdrawing the Holding of Abandonment When Office Actions Are Not Received," 1156 O.G. 53 (November 16, 1993).

The showing outlined above may not be sufficient if there are circumstances that point to a conclusion that the Office action may have been lost after receipt rather than a conclusion that the Office action was lost in the mail (e.g., if the practitioner has a history of not receiving Office actions).

Evidence of nonreceipt of an Office communication or action (e.g., Notice of Abandonment or an advisory action) other than that action to which reply was required to avoid abandonment would not warrant withdrawal of the holding of abandonment. Abandonment takes place by operation of law for failure to reply to an Office action or timely pay the issue fee, not by operation of the mailing of a Notice of Abandonment. See Lorenz v. Finkl, 333 F.2d 885, 889-90, 142 USPQ 26, 29-30 (CCPA 1964); Krahn v. Commissioner, 15 USPQ2d 1823, 1824 (E.D. Va 1990); In re Application of Fischer, 6 USPQ2d 1573, 1574 (Comm'r Pat. 1988).

ANALYSIS

Regarding Petitioner's request that the declaration be considered to have been timely filed, this request cannot be granted, for the following reasons.

Petitioner has submitted a declaration of facts which asserts that a partner in the Petitioner's law firm has reviewed the docket report, and that the notice was not received. First, it is noted that the declarant states that he has reviewed the docket report, but he does not set forth that the notice does not appear on the docket report. Secondly, the statement does not make any reference to any search of the file jacket which corresponds to the instant application. Third, the declarant has not set forth that he has searched the place where this notice would normally be kept, had it been received. Fourth, a copy of the docket record where the non-received notice would have been entered had it been received and docketed has not been attached to the declarant's statement. Fifth, Petitioner has not included a copy of the file jacket associated with this application.

Even if Petitioner were to establish that the notice was not received, the executed declaration would not be considered timely filed, as the executed declaration was due on filing. The application was filed on June 23, 2003, and the executed declaration was not submitted until almost eight months later. A review of the electronic record shows that the declaration submitted on filing was not an executed declaration. As such, an acceptable declaration was not timely filed, and the Office will not determine that a declaration was properly submitted on filing when it is clear that such is not the case.

Regarding the waiver of the petition fee, the request cannot be granted, as petition fees are jurisdictional. Petitioner will note that the last sentence of 37 C.F.R. §1.182 specifically sets forth "Any petition seeking a decision under this section must be accompanied by the petition fee set forth in § 1.17(h)." As such, the petition fee of $130 has been charged to Petitioner's Deposit Account, as authorized in the petition, along with the surcharge associated with the late submission of an oath or declaration.

The declaration submitted with the instant petition will not be entered until either the petition under 37 C.F.R. §.182 is granted or the Petitioner corrects the deficiencies noted in the second paragraph of this section of the decision.

CONCLUSION

For these reasons, the petition under 37 CFR 1.182 is dismissed.

Petitioner is given **TWO MONTHS** from the mailing date of this decision to reply, correcting the above-noted deficiencies. Any reply should be entitled "Renewed Petition Under 37 C.F.R. §1.182," and should only address the deficiencies noted above. **Failure to respond will result in abandonment of the application**.

Any extensions of time will be governed by 37 C.F.R. §1.136(a). Extensions of time under 37 CFR §1.136(a) are permitted. The reply should include a cover letter entitled "Renewed Petition Under 37 CFR §1.182". This is not a final agency action within the meaning of 5 U.S.C 704.

Any renewed petition may be submitted by mail[1], hand-delivery[2], or facsimile[3].

The reply should display "Please deliver to Paul Shanoski, c/o Office of Petitions" in a prominent manner. The Petitioner may wish to consider telephoning the undersigned at the number provided below to confirm that the documents were delivered to the undersigned. Please note that the delivery process within the PTO can take as much as three weeks.

The application file will be retained in the Office of Petitions for two (2) months.

---

1 Mail Stop Petition, Commissioner for Patents, United States Patent and Trademark Office, P.O. Box 1450, Alexandria, VA, 22313-1450.

2 Customer Window, Mail Stop Petition, Crystal Plaza Two, Lobby, Room 1B03, Arlington, Virginia 22202.

3 (703) 872-9306 - please note this is a central facsimile number, and as such, there will be a delay in the delivery of the facsimile to the undersigned.

Telephone inquiries regarding this decision should be directed to the undersigned at (703) 305-0011.

Paul Shanoski
Senior Attorney
Office of Petitions
United States Patent and Trademark Office

United States Patent and Trademark Office

PATENT APPLICATION INFORMATION RETRIEVAL

Search results for application number:10/602,394

Application Number:	10/602,394	Customer Number:	23557
Filing or 371(c) Date:	06-23-2003	Status:	Application Dispatche Preexam, Not Yet Dock
Application Type:	Utility	Status Date:	03-24-2004
Examiner Name:	-	Location:	ELECTRONIC
Group Art Unit:	1646	Location Date:	07-21-2003
Confirmation Number:	1696	Earliest Publication No:	-
Attorney Docket Number:	UF-375	Earliest Publication Date:	-
Class/ Sub-Class:	530/-	Patent Number:	-
First Named Inventor:	Carrie Haskell-Luevano, Archer, FL (US)	Issue Date of Patent:	-
Title Of Invention:	Novel melanocortin receptor templates, peptides, and use there		

Select Search Option

Image File Wrapper | Search

Publication Review

File History

Number	Date	Contents Description
13	02-13-2004	Petition Entered
12	03-24-2004	Application Return from OIPE
11	03-24-2004	Application Return TO OIPE
10	03-24-2004	Application Dispatched from OIPE
9	03-24-2004	Application Is Now Complete
8	02-13-2004	Additional Application Filing Fees
7	02-13-2004	A statement by one or more inventors satisfying the requireme USC 115, Oath of the Applic
6	09-08-2003	Notice Mailed--Application Incomplete--Filing Date Assigned
5	08-20-2003	Cleared by L&R (LARS)
3	08-19-2003	Referred to Level 2 (LARS) by OIPE CSR
2	07-15-2003	IFW Scan & PACR Auto Security Review
1	06-23-2003	Initial Exam Team nn

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United States Patent and Trademark Office
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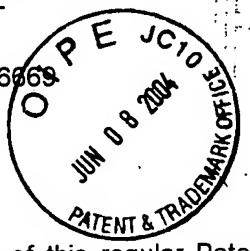
APPL NO.	FILING OR 371 (c) DATE	ART UNIT	FIL FEE REC'D	ATTY.DOCKET NO	DRAWINGS	TOT CLMS	IND CLMS
10/602,394	06/23/2003	1646	755	UF-375	1	27	9

CONFIRMATION NO. 1696

UPDATED FILING RECEIPT

OC000000012173686

23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
2421 N.W. 41ST STREET
SUITE A-1
GAINESVILLE, FL 326066669



MAR 27 2004

Date Mailed: 03/24/2004

Receipt is acknowledged of this regular Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Filing Receipt Corrections, facsimile number 703-746-9195. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

When did we receive the OFR?

Applicant(s)

Carrie Haskell-Luevano, Archer, FL;

Domestic Priority data as claimed by applicant

Foreign Applications

If Required, Foreign Filing License Granted: 09/08/2003

Projected Publication Date: 12/23/2004

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Novel melanocortin receptor templates, peptides, and use thereof

Preliminary Class

530

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Office of Export Administration, Department of Commerce (15 CFR 370.10 (j)); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

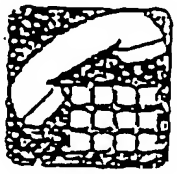
NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

U.S. Department of Commerce

Patent and Trademark Office

Office of Initial Patent Examination (OIPE)



DATE: 01-16-04

SEND TO: Ms Linda

Fax Number: 352-372-5800

Office Telephone Number:

FROM: Preston Wallace

Fax Number:

Office Telephone Number: 703-308-9452

Pages Sent (Including cover sheet) 7

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
10/602,394	06/23/2003	Carrie Haskell-Luevano	UF-375

CONFIRMATION NO. 1696

23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
2421 N.W. 41ST STREET
SUITE A-1
GAINESVILLE, FL 326066669

FORMALITIES LETTER

OC000000010840623

Date Mailed: 09/08/2003

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The oath or declaration is unsigned.
- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(e) of $65 for a small entity in compliance with 37 CFR 1.27, must be submitted with the missing items identified in this letter.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$65** for a Small Entity

- **$65** Late oath or declaration Surcharge.

Replies should be mailed to: Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

A copy of this notice MUST be returned with the reply.

Customer Service Center
Initial Patent Examination Division (703) 308-1202
PART 1 - ATTORNEY/APPLICANT COPY

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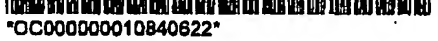
APPL NO.	FILING OR 371 (c) DATE	ART UNIT	FIL FEE REC'D	ATTY.DOCKET NO	DRAWINGS	TOT CLMS	IND CLMS
10/602,394	06/23/2003	1646	690	UF-375	1	27	9

CONFIRMATION NO. 1696

23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
2421 N.W. 41ST STREET
SUITE A-1
GAINESVILLE, FL 326066669

FILING RECEIPT



"OC000000010840622"

Date Mailed: 09/08/2003

Receipt is acknowledged of this regular Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Filing Receipt Corrections, facsimile number 703-746-9195. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Carrie Haskell-Luevano, Archer, FL;

Domestic Priority data as claimed by applicant

Foreign Applications

If Required, Foreign Filing License Granted: 09/08/2003

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Novel melanocortin receptor templates, peptides, and use thereof

Preliminary Class

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Office of Export Administration, Department of Commerce (15 CFR 370.10 (j)); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Petitions, Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on February 9, 2004.

Margaret Efron, Patent Attorney

Examining Group 1646
Patent Application
Docket No. UF-375
Serial No. 10/602,394
Conf. No. 1696

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Carrie Haskell-Luevano
Serial No.	:	10/602,394
Filed	:	June 23, 2003
Art Unit	:	1646
For	:	Novel Melanocortin Receptor Templates, Peptides, and Use Thereof
Conf. No.	:	1696

Mail Stop PETITION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

PETITION UNDER 37 CFR 1.182

Sir:

The above-referenced patent application was filed with an unsigned Declaration (37 CFR 1.63) and Power of Attorney form. Transmitted herewith is a fully executed Declaration (37 CFR 1.63) and Power of Attorney form for the subject application.

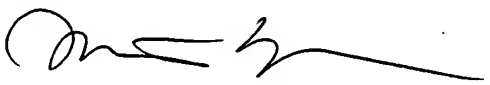
With regard to the timing of this submission, please note that, upon a routine review of our docket on January 16, 2004, it was determined that our firm had never received a Notice to File Missing Parts in this case. From a review of PAIR on January 16, 2004, it was noted that a Notice to File Missing Parts of Nonprovisional Application dated September 8, 2003 had been mailed. Consultation with personnel at the Notice to File Missing Parts Office resulted in receipt of the missing Notice by facsimile (after an erroneous transmittal first to the law firm of Wechsler & Wechsler) on January 16, 2004. A copy of that Notice is attached hereto.

The applicant respectfully submits that a Notice to File Missing Parts of Nonprovisional Application for the above-referenced application was not received by the applicant during the month of September 2003 or thereafter, until January 16, 2004. Attached herewith is a Declaration of Mr. David R. Saliwanchik, stating that all incoming mail records have been checked and that no such Notice regarding the subject application was received from September 2003 through January 15, 2004.

The applicant hereby respectfully petitions that the submission of the executed Declaration and Power of Attorney be considered timely, and that a Petition fee should not be charged (or should be refunded if it is charged).

To the extent that a Petition fee is necessary, the fee of $130 set forth in 37 CFR 1.17(h) should be charged to Deposit Account No. 19-0065. Please charge the surcharge of $65.00 (for the late-filed declaration) to Deposit Account No. 19-0065. The Commissioner is hereby authorized to charge any additional fees that may be required to Deposit Account No. 19-0065. Two copies of this petition are enclosed.

Respectfully submitted,



Margaret H. Efron
Patent Attorney
Registration No. 47,545
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: 2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669

MHE/la

Attachments: Executed Declaration and Power of Attorney form;
Two copies of this Petition and authorization to deposit account;
Copy of Notice to File Missing Parts of Nonprovisional Application received January 16, 2004; and
Declaration of David R. Saliwanchik under 37 CFR §1.131.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	:	1646
Applicant(s)	:	Carrie Haskell-Luevano
Serial No.	:	10/602,394
Conf. No.	:	1696
Filed	:	June 23, 2003
For	:	Novel Melanocortin Receptor Templates, Peptides, and Use Thereof

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DAVID R. SALIWANCHIK UNDER 37 CFR §1.131

Sir:

I, DAVID R. SALIWANCHIK, hereby declare:

THAT, I am a partner in the law firm of Saliwanchik, Lloyd & Saliwanchik (SLS);

THAT, the attorneys of SLS are the attorneys of record on the above-referenced patent application;

THAT, each piece of incoming mail received by SLS is recorded by our docketing department, and entered into our computerized docket system;

THAT, I have reviewed a list of all of the incoming correspondence received by our firm for September 2003 through January 15, 2004; and

Being thus duly qualified, do further declare as follows:

Prior to January 16, 2004, a Notice to File Missing Parts of Nonprovisional Application for U.S. Patent Application Serial No. 10/602,394 was not received by the law firm of Saliwanchik, Lloyd & Saliwanchik.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By: David Saliwanchik
David R. Saliwanchik

Feb. 9, 2004
Date

DECLARATION (37 C.F.R. § 1.63) AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name; and

I believe that I am the original, first, and sole inventor (if only one name is listed below), or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **Novel Melanocortin Receptor Templates, Peptides, and Use Thereof** , specification for which

☐ is attached hereto.
☒ was filed June 23, 2003 , Serial No. 10/602,394 .

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 and/or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Application Serial No.	Country	Filing Date	Priority Claimed

I hereby claim priority benefits under Title 35, United States Code §119 of any provisional application(s) for patent listed below:

Application Serial No.	Filing Date	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, §120 and/or §365 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (Patented, Pending, Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following persons registered to practice before the Patent and Trademark Office as my attorneys with full power of substitution and revocation to prosecute this application and all divisions and continuations thereof and to transact all business in the Patent and Trademark Office connected therewith: John M. Sanders, Reg. No. 30,126; David R. Saliwanchik, Reg. No. 31,794; Jeff Lloyd, Reg. No. 35,589; Doran R. Pace, Reg. No. 38,261; Jay M. Sanders, Reg. No. 39,355; Jean Kyle, Reg. No. 36,987; James S. Parker, Reg. No. 40,119; Frank C. Eisenschenk, Reg. No. 45,332; Glenn P. Ladwig, Reg. No. 46,853; Margaret Efron, Reg. No. 47,545; and Gwendolyn L. Daniels, Reg. No. 51,594.

I request that all correspondence be sent to:

Margaret Efron
Saliwanchik, Lloyd & Saliwanchik
A Professional Association
2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669

I further request that all telephone communications be directed to:

Margaret Efron
352-375-8100

Name of First or Sole Inventor: Carrie Haskell-Luevano

Residence: Archer, FL Citizenship: United States

Post Office Address: 10406 S.W. CR 346
Archer, FL 32618 USA

Signature of First or Sole Inventor Date: 7/22/03

Form PTO-1595
(Rev. 03/01)
OMB No. 0651-0027 (exp. 5/31/2002)

RECORDATION FORM COVER SHEET
PATENTS ONLY

U.S. DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office

Tab Settings ⇨ ⇨ ⇨ ▼ ▼ ▼ ▼ ▼ ▼ ▼

To the Honorable Commissioner of Patents and Trademarks: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):

1) Carrie Haskell-Luevano

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Name and address of receiving party(ies)

Name: University of Florida

Internal Address:

Street Address: 223 Grinter Hall

Gainesville, FL 32611

Additional name(s) & address(es) attached? ☐ Yes ☒ No

3. Nature of conveyance:

☒ Assignment ☐ Merger
☐ Security Agreement ☐ Change of Name
☐ Other

Execution Date: July 22, 2003

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is:

A. Patent Application No.(s)

SN 10/602,394; filed June 23, 2003

B. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Margaret Efron

Internal Address: Saliwanchik, Lloyd & Saliwanchik

A Professional Association

Street Address: 2421 N.W. 41st Street, Suite A-1

City: Gainesville State: FL Zip: 32606

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41)..... $ 40.00

☐ Enclosed

☒ Authorized to be charged to deposit account

8. Deposit account number:

19-0065

(Attach duplicate copy of this page if paying by deposit account)

DO NOT USE THIS SPACE

9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Margaret Efron
Name of Person Signing

Signature

September 16, 2003
Date

Total number of pages including cover sheet, attachments, and documents: 3 Atty Docket No. UF-375

Mail documents to be recorded with required cover sheet information to:
Commissioner of Patents & Trademarks, Box Assignments
Washington, D.C. 20231

Docket No. UF-375

ASSIGNMENT

WHEREAS, I, the undersigned, residing at the indicated address given below, have invented certain new and useful improvements in **Novel Melanocortin Receptor Templates and Use Thereof,** for which an application for United States Letters Patent was

❑ signed by us as dated below.

☒ filed June 23, 2003; Serial No. 10/602,394

WHEREAS, the UNIVERSITY OF FLORIDA, existing by virtue of the laws of the State of Florida, and having an office at 223 Grinter Hall, Gainesville, Florida 32611, is desirous of acquiring the entire right, title and interest in and to said invention and in and to any Letters Patent which may be granted therefor in the United States and in any and all foreign countries;

NOW, THEREFORE, in view of my prior employment with the UNIVERSITY OF FLORIDA, and other valuable consideration, I, the undersigned, have sold, assigned, and transferred, and by these presents do sell, assign, and transfer, unto said UNIVERSITY OF FLORIDA, its successors and assigns, the full and exclusive right to the said invention in the United States and its territorial possessions and in all foreign countries and the entire right, title, and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations, and extensions thereof.

I hereby authorize and request the Patent Office Officials in the United States and in any and all foreign countries to issue any and all of said Letters Patent, when granted, to said UNIVERSITY OF FLORIDA, as the assignee of the entire right, title and interest in and to the same, for the sole use and behoof of said UNIVERSITY OF FLORIDA, its successors and assigns.

FURTHER, I agree that we will communicate to said UNIVERSITY OF FLORIDA, or its representatives, any facts known to me respecting said invention; testify in any legal proceedings; sign all lawful papers; execute all divisional, continuation, substitution, renewal, and reissue applications; execute all necessary assignment papers to cause any and all of said Letters Patent to be issued to said UNIVERSITY OF FLORIDA; make all rightful oaths; and generally do everything possible to aid the said UNIVERSITY OF FLORIDA, its successors and assigns, to obtain and enforce proper protection for said invention in the United States and in any and all foreign countries.

IN TESTIMONY WHEREOF, I have hereunto set my hand this 22 day of July, ~~2002~~ 2003 CR

Signed _____
Carrie Haskell-Luevano

Address: 10406 S.W. CR 346
Archer, FL 32618

State of Florida)

County of Alachua)

On this 24th day of July, 2003 personally appeared before me the above-named CARRIE HASKELL-LUEVANO, to me known to me to be the person described in the foregoing instrument, who executed the foregoing instrument, and who acknowledged the same to be his free act and deed in and for the purposes set forth in said instrument.

_____
Notary Public LYNNE B. COLLINS

My Commission Expires: July 9, 2007

SEAL

LYNNE B. COLLINS
MY COMMISSION # DD 230370
EXPIRES: July 9, 2007
Bonded Thru Budget Notary Services

Type of Identification: Personally Known

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Mail Stop DD, Commissioner for Patents, Alexandria, VA 22313 on the date shown below:

August 28, 2003

Margaret H. Erfron, Patent Attorney

INFORMATION DISCLOSURE STATEMENT
Patent Application
Docket No. UF-375
Serial No. 10/602,394

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	:	(not yet assigned)
Applicant(s)	:	Carrie Haskell-Luevano
Serial No.	:	10/602,394
Filed	:	June 23, 2003
Conf. No.	:	(not yet assigned)
For	:	Novel Melanocortin Receptor Templates, Peptides, and Use Thereof

Mail Stop DD
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 C.F.R. §§1.97 AND 1.98

Sir:

In accordance with 37 C.F.R. §1.56, the references listed on the attached form PTO/SB/08 are being brought to the attention of the Examiner for consideration in connection with the examination of the above-identified patent application. Copies of the cited documents are enclosed.

The applicant respectfully asserts that the substantive provisions of 37 C.F.R. §§1.97 and 1.98 are met by the foregoing statement.

Respectfully submitted,

Margaret H. Efron
Patent Attorney
Registration No. 47,545
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: 2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669

MHE/ba
Attachments: Form PTO/SB/08 (3 pages) and references listed thereon (32 refs.).

S:\HOME\AudetteL\DOC\IDS\UF\UF-375-pto.doc\DNB/ba

PTO/SB/08A (10-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet	1	of	3

Complete if Known	
Application Number	10/602,394
Filing Date	June 23, 2003
First Named Inventor	Carrie Haskell-Luevano
Art Unit	(not yet assigned)
Examiner Name	(not yet assigned)
Attorney Docket Number	UF-375

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No. [1]	Document Number Number - Kind Code [2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	U1	US-6,127,381	10-03-2000	Basu *et al.*	All
	U2	US-6,451,783 B1	09-17-2002	Hadcock *et al.*	All
	U3	US-			
	U4	US-			
	U5	US-			
	U6	US-			
	U7	US-			
	U8	US-			
	U9	US-			
	U10	US-			
	U11	US-			
	U12	US-			
	U13	US-			
	U14	US-			
	U15	US-			
	U16	US-			
	U17	US-			
	U18	US-			
	U19	US-			
	U20	US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No. [1]	Foreign Patent Document Country Code [3]	Number [4]	Kind Code [5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T [6]
	F1	WO	01/74844	A2	10-11-2001	F. Hoffmann-La Roche Ag	All	
	F2	WO	02/18437	A2	03-07-2002	F. Hoffmann-La Roche Ag	All	
	F3	WO	03/006620	A2	01-23-2003	Palatin Technologies, Inc.	All	
	F4	WO	99/21571	A1	05-06-1999	Trega Biosciences, Inc.	All	
	F5	WO	99/54358	A1	10-28-1999	Quadrant Holdings Cambridge Limited	All	
	F6							
	F7							
	F8							
	F9							
	F10							

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] See Kind Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08B (10-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449B/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 2 of 3

Complete if Known	
Application Number	10/602,394
Filing Date	June 23, 2003
First Named Inventor	Carrie Haskell-Luevano
Group Art Unit	(not yet assigned)
Examiner Name	(not yet assigned)
Attorney Docket Number	UF-375

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article, (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	R1	BOLIN, K.A. *et al.* "NMR Structure of a Minimized Human Agouti Related Protein Prepared by Total Chemical Synthesis" *FEBS Letters*, 1999, pp. 125-131, Vol. 451.	
	R2	CASTRUCCI, A.M.L. *et al.* "α-Melanotropin: The Minimal Active Sequence in the Lizard Skin Bioassay" *General and Comparitive Endocrinology*, 1989, pp. 157-163, Vol. 73.	
	R3	HRUBY, V.J. *et al.* "α-Melanotropin: The Minimal Active Sequence in the Frog Skin Bioassay" *J. Med. Chem.*, 1987, pp. 2126-2130, Vol. 30.	
	R4	HOLDER, J. R. *et al.* "Structure-Activity Relationships of the Melanocortin Tetrapeptide Ac-His-DPhe-Arg-Trp-NH_2 at the Mouse Melanocortin Receptors. 1. Modifications at the His Position" *J. Med. Chem.*, 2002, pp. 2801-2810, Vol. 45.	
	R5	HOLDER, J. R. *et al.* "Structure-Activity Relationships of the Melanocortin Tetrapeptide Ac-His-DPhe-Arg-Trp-NH_2 at the Mouse Melanocortin Receptors: Part 2 Modifications at the Phe Position" *J. Med. Chem.*, 2002, pp. 3073-3081, Vol. 45.	
	R6	JACKSON, P. J. *et al.* "Design, Pharmacology, and NMR Structure of a Minimized Cystine Knot with Agouti-Related Protein Activity" *Biochemistry*, 2002, pp.7565-7572, Vol. 41. No. 24.	
	R7	KAVARANA, M. J. *et al.* "Novel Cyclic Templates of α-MSH Give Highly Selective and Potent Antagonists/ Agonists for Human Melanocortin-3/4 Receptors" *J. Med. Chem.*, 2002, pp. 2644-2650, Vol. 45.	
	R8	KIEFER, L. L. *et al.* "Melanocortin Receptor Binding Determinants in the Agouti Protein" *Biochemistry*, 1998, pp. 991-997, Vol. 37.	
	R9	KIEFER, L. L. *et al.* "Mutations in the Carboxyl Terminus of the Agouti Protein Decrease Agouti Inhibition of Ligand Binding to the Melanocortin Receptors" *Biochemistry*, 1997, pp. 2084-2090, Vol. 36.	
	R10	KIM *et al.*, "Hypothalamic Localization of the Feeding Effect of Agouti-Related Peptide and α-Melanocyte-Stimulating Hormone," *Diabetes*, February 2000, pp. 177-182, Vol. 49.	
	R11	HASKELL-LUEVANO, C. *et al.* "Characterization of Melanocortin NDP-MSH Agonist Fragments at the Mouse Central and Peripheral Melanocortin Receptors" *J. Med. Chem.*, 2001, pp. 2247-2252, Vol. 44.	
	R12	HASKELL-LUEVANO, C. *et al.* "The Agouti-Related Protein Decapeptide (Yc[CRFFNAFC]Y) Possesses Agonist Activity at the Murine Melanocortin-1 Receptor" *Peptides*, 2000, pp. 683-689, Vol. 21.	
	R13	HASKELL-LUEVANO, C. *et al.* "Structure Activity Studies of the Melanocortin-4 Receptor by *in Vitro* Mutagenesis: Identification of Agouti-Related Protein (AGRP), Melanocortin Agonist and Synthetic Peptide Antagonist Interaction Determinants" *Biochemistry*, 2001, pp. 6164-6179, Vol. 40.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08B (10-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449B/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 3 of 3

Complete if Known	
Application Number	10/602,394
Filing Date	June 23, 2003
First Named Inventor	Carrie Haskell-Luevano
Group Art Unit	(not yet assigned)
Examiner Name	(not yet assigned)
Attorney Docket Number	UF-375

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article, (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	R14	McNulty, J. C. *et al.* "High-Resolution NMR Structure of the Chemically-Synthesized Melanocortin Receptor Binding Domain AGRP(87-132) of the Agouti-Related Protein" *Biochemistry,* 2001, pp. 15520-15527. Vol. 40.	
	R15	AL-OBEIDI, F. *et al.* "Potent and Prolonged Acting Cyclic Lactam Analogues of α-Melanotropin: Design Based on Molecular Dynamics" *J. Med. Chem.* 1989, pp. 2555-2561, Vol. 32.	
	R16	OOSTEROM, J. *et al.* "Common Requirements for Melanocortin-4 Receptor Selectivity of Structurally Unrelated Melanocortin Agonist and Endogenous Antagonist, Agouti Protein" *The Journal of Biological Chemistry*, January 12, 2001, pp. 931-936, Vol. 276, No. 2.	
	R17	PERRY, W. L. *et al.* "A Transgenic Mouse Assay for Agouti Protein Activity" *Genetics*, May 1995, pp. 267-274, Vol. 140.	
	R18	PERRY, W. L. *et al.* "Coupled Site-Directed Mutagenesis/Transgenesis Identifies Important Functional Domains of the Mouse Agouti Protein" *Genetics*, September 1996, pp. 255-264, Vol. 144.	
	R19	QUILLAN, J. M. *et al.* "A Synthetic Human Agouti-Related Protein-(83-132)-NH_2 Fragment is a Potent Inhibitor of Melanocortin Receptor Function" *FEBS Letters*, 1998, pp. 59-62, Vol. 428.	
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	R21	TOTA, M. R. *et al.* "Molecular Interaction of Agouti Protein and Agouti-Related Protein with Human Melanocortin Receptors" *Biochemistry*, 1999, pp. 897-904, Vol. 38.	
	R22	WILLARD, D. H. *et al.* "Agouti Structure and Function: Characterization of a Potent α-Melanocyte Stimulating Hormone Receptor Antagonist" *Biochemistry,* 1995, pp. 12341-12346, Vol. 34.	
	R23	YANG, Y-K. *et al.* "Functional Properties of an Agouti Signaling Protein Variant and Characteristics of its Cognate Radioligand" *Am. J. Physiol Regulatory Integrative Comp. Physiol.*, 2001, pp. R1877-R1886, Vol. 281.	
	R23	YANG, Y-K. *et al.* "Molecular Determinants of Ligand Binding to the Human Melanocortin-4 Receptor" *Biochemistry*, 2000, pp. 14900-14911, Vol. 39.	
	R25	YANG, Y-K. *et al.* "Characterization of Agouti-Related Protein Binding to Melanocortin Receptors" *Molecular Endocrinology,* 1999, pp. 148-155.	
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	UF-375
First Inventor	Carrie Haskell-Luevano
Title	Novel Melanocortin Receptor Templates, Peptides, and Use Thereof
Express Mail Label No.	EU 082849555 US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

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   - Descriptive title of the invention
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   - Reference to sequence listing, a table, or an appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
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Claims

I claim:

1. A peptide that is biologically active at melanocortin receptors comprising an AGRP(109-118) template and melanocortin agonist-based bioactive determinant sequences which have been substituted for the analogous template sequences, wherein

a) the melanocortin agonist-based bioactive determinant sequence is selected from the group consisting of:

i) Trp-Arg-Phe;

ii) Trp-Arg-DPhe;

iii) Phe-Arg-Trp;

iv) DPhe-Arg-Trp;

v) His-Phe-Arg-Trp; and

vi) His-DPhe-Arg-Trp.

2. The peptide according to claim 1, wherein the peptide is of any SEQ ID NOS:4-7, 9, and 10.

3. The peptide according to claim 1, wherein the melanocortin agonist-based bioactive determinant sequence includes at least one amino acid substituted within the sequence.

4. The peptide according to claim 3, wherein the amino acid is selected from the group consisting of Ala; Atc; Bip; Lys; Nal(1'); Nal(2'); (pI)Phe; and Tic.

5. The peptide according to claim 4, wherein the peptide is of any SEQ ID NOS:24-43.

6. The peptide according to claim 1, wherein the peptide further comprises a lactam bridge which is substituted for the disulfide bridge of the AGRP(109-118) template.

7. The peptide according to claim 6, wherein the peptide is of any SEQ ID NOS:2 and 11.

8. The peptide according to claim 6, wherein the peptide further comprises a second and a third bioactive determinant sequences at the N-terminal and C-terminal, respectively, wherein the second bioactive determinant sequence at the N-terminal is Ser-Tyr-Ser-Nle amino acid residues and the third bioactive determinant sequence at the C-terminal is Lys-Pro-Val amino acid residues.

9. A peptide that is biologically active at melanocortin receptors comprising a NDP-MSH linear tridecapeptide template and hAGRP(111-113) bioactive determinant sequences which have been substituted for the analogous template sequences, wherein

a) the hAGRP(111-113) bioactive determinant sequence is selected from the group consisting of:

i) Arg-Phe-Phe;

ii) Phe-Phe-Arg;

iii) DArg-Phe-Phe;

iv) Arg-DPhe-Phe; and

v) Arg-Phe-DPhe.

10. The peptide according to claim 9, wherein the peptide is of any SEQ ID NOS:13-18.

11. A peptide that is biologically active at melanocortin receptors comprising a cyclic MTII heptapeptide template and hAGRP(111-113) bioactive determinant sequences which have been substituted for the analogous template sequences, wherein

a) the hAGRP(111-113) bioactive determinant sequence is selected from the group consisting of:

i) Arg-Phe-Phe;

ii) Phe-Phe-Arg;

iii) DArg-Phe-Phe;

iv) Arg-DPhe-Phe; and

v) Arg-Phe-DPhe.

12. The peptide according to claim 11, wherein the peptide is of any SEQ ID NOS:20-23.

13. A pharmaceutical composition comprising a peptide that is biologically active at melanocortin receptors comprising an AGRP(109-118) template and melanocortin agonist-based bioactive determinant sequences which have been substituted for the analogous template sequences, and a pharmaceutically acceptable carrier or diluent, wherein

a) the melanocortin agonist-based bioactive determinant sequence is selected from the group consisting of:

i) Trp-Arg-Phe;

ii) Trp-Arg-DPhe;

iii) Phe-Arg-Trp;

iv) DPhe-Arg-Trp;

v) His-Phe-Arg-Trp; and

vi) His-DPhe-Arg-Trp.

14. The pharmaceutical composition according to claim 13, wherein the peptide is of any SEQ ID NOS:4-7, 9, and 10.

15. The pharmaceutical composition according to claim 13, wherein the melanocortin agonist-based bioactive determinant sequence includes at least one amino acid substituted within the sequence.

16. The pharmaceutical composition according to claim 15, wherein the amino acid is selected from the group consisting of Ala; Atc; Bip; Lys; Nal(1'); Nal(2'); (pI)Phe; and Tic.

17. The pharmaceutical composition according to claim 16, wherein the peptide is of any SEQ ID NOS:24-43.

18. The pharmaceutical composition according to claim 13, wherein the peptide further comprises a lactam bridge which is substituted for the disulfide bridge of the AGRP(109-118) template.

19. The pharmaceutical composition according to claim 18, wherein the peptide is of any SEQ ID NOS:2 and 11.

20. The pharmaceutical composition according to claim 18, wherein the peptide further comprises a second and a third bioactive determinant sequences at the N-terminal and C-terminal, respectively, wherein the second bioactive determinant sequence at the N-terminal is Ser-Tyr-Ser-Nle amino acid residues and the third bioactive determinant sequence at the C-terminal is Lys-Pro-Val amino acid residues.

21. A pharmaceutical composition comprising a peptide that is biologically active at melanocortin receptors comprising a NDP-MSH linear tridecapeptide template and hAGRP(111-113) bioactive determinant sequences which have been substituted for the analogous template sequences, and a pharmaceutically acceptable carrier or diluent, wherein

a) the hAGRP(111-113) bioactive determinant sequence is selected from the group consisting of:

i) Arg-Phe-Phe;

ii) Phe-Phe-Arg;

iii) DArg-Phe-Phe;

iv) Arg-DPhe-Phe; and

v) Arg-Phe-DPhe.

22. The pharmaceutical composition according to claim 21, wherein the peptide is of any SEQ ID NOS:13-18.

23. A pharmaceutical composition comprising a peptide that is biologically active at melanocortin receptors comprising a cyclic MTII heptapeptide template and hAGRP(111-113) bioactive determinant sequences which have been substituted for the analogous template sequences, and a pharmaceutically acceptable carrier or diluent, wherein

a) the hAGRP(111-113) bioactive determinant sequence is selected from the group consisting of:

i) Arg-Phe-Phe;

ii) Phe-Phe-Arg;

iii) DArg-Phe-Phe;

iv) Arg-DPhe-Phe; and

v) Arg-Phe-DPhe.

24. The pharmaceutical composition according to claim 23, wherein the peptide is of any SEQ ID NOS:20-23.

25. A method for treating in a patient a condition modulated by melanocortin receptors, the method comprising administering to the patient a pharmaceutical composition comprising a peptide that is biologically active at melanocortin receptors

comprising an AGRP(109-118) template and melanocortin agonist-based bioactive determinant sequences which have been substituted for the analogous template sequences, and a pharmaceutically acceptable carrier or diluent, wherein

a) the melanocortin agonist-based bioactive determinant sequence is selected from the group consisting of:

i) Trp-Arg-Phe;

ii) Trp-Arg-DPhe;

iii) Phe-Arg-Trp;

iv) DPhe-Arg-Trp;

v) His-Phe-Arg-Trp; and

vi) His-DPhe-Arg-Trp.

26. A method for treating in a patient a condition modulated by melanocortin receptors, the method comprising administering to the patient a pharmaceutical composition comprising a peptide that is biologically active at melanocortin receptors comprising a NDP-MSH linear tridecapeptide template and hAGRP(111-113) bioactive determinant sequences which have been substituted for the analogous template sequences, and a pharmaceutically acceptable carrier or diluent, wherein

a) the hAGRP(111-113) bioactive determinant sequence is selected from the group consisting of:

i) Arg-Phe-Phe;

ii) Phe-Phe-Arg;

iii) DArg-Phe-Phe;

iv) Arg-DPhe-Phe; and

v) Arg-Phe-DPhe.

27. A method for treating in a patient a condition modulated by melanocortin receptors, the method comprising administering to the patient a pharmaceutical composition comprising a peptide that is biologically active at melanocortin receptors comprising a cyclic MTII heptapeptide template and hAGRP(111-113) bioactive

determinant sequences which have been substituted for the analogous template sequences, and a pharmaceutically acceptable carrier or diluent, wherein

a) the hAGRP(111-113) bioactive determinant sequence is selected from the group consisting of:

i) Arg-Phe-Phe;

ii) Phe-Phe-Arg;

iii) DArg-Phe-Phe;

iv) Arg-DPhe-Phe; and

v) Arg-Phe-DPhe.

Abstract

The present invention relates to novel chimeric peptides and templates containing a combination of antagonist and agonist endogenous ligand residues. In particular, the present invention relates to novel chimeric peptides and templates thereof based upon melanocortin agonist peptides and agouti related protein (AGRP). The present invention provides multifunctional chimeric peptides having specific bioactivity at melanocortin receptors and their use as drugs to treat various diseases and conditions.

Peptide	Primary sequence
α-MSH	Ac-Ser-Tyr-Ser-Met-Glu-His-**Phe-Arg-Trp**-Gly-Lys-Pro-Val-NH_2
NDP-MSH	Ac- Ser-Tyr-Ser -Nle- Glu-His-**DPhe-Arg-Trp**-Gly-Lys-Pro-Val -NH_2
MT-II	Ac-Nle-c[Asp-His-**DPhe-Arg-Trp**-Lys]-NH_2
hAGRP (87-132)	CVRLHESCLGQQVPCCDPCATCYC**RFF**NAFCYCRKLGTAMNPCSRT

FIG. 1

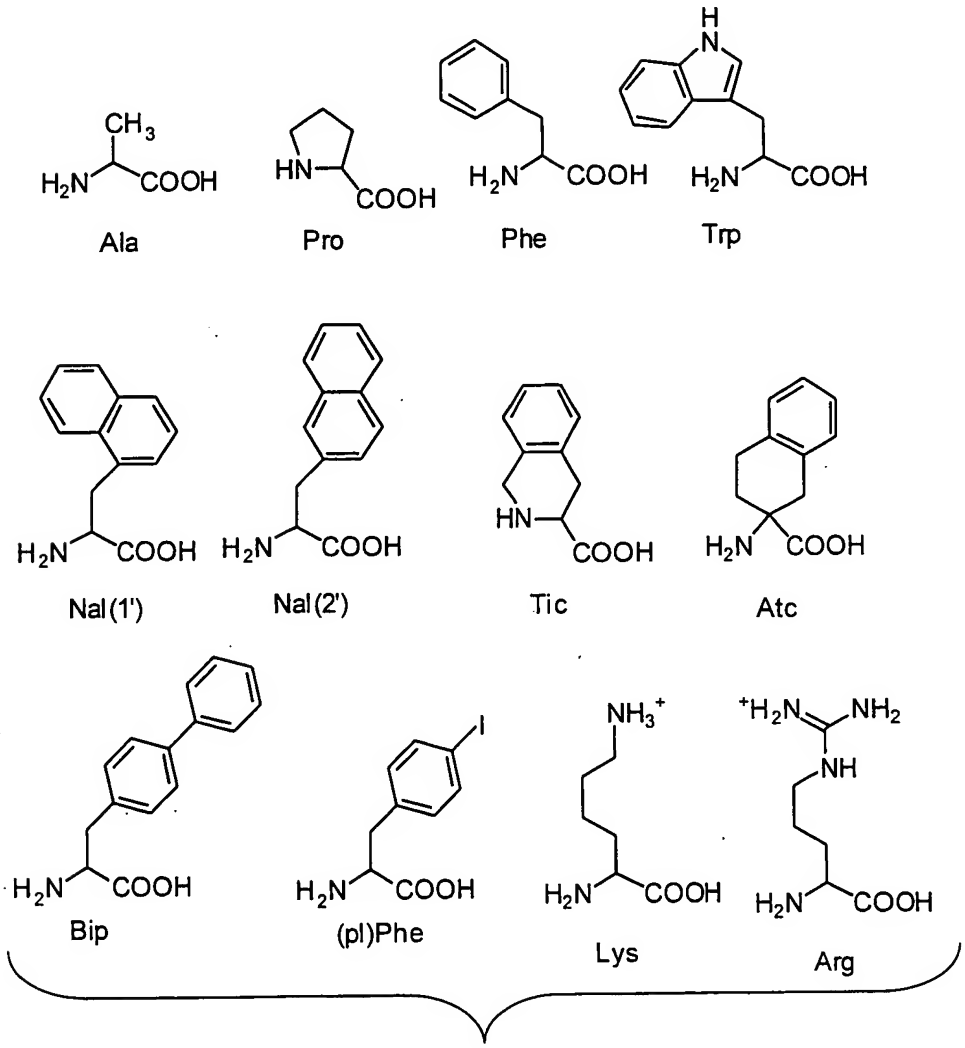

FIG. 2

Peptide	Primary sequence
α-MSH	Ac-Ser-Tyr-Ser-Met-Glu-His-**Phe-Arg-Trp**-Gly-Lys-Pro-Val-NH_2
NDP-MSH	Ac- Ser-Tyr-Ser -Nle- Glu-His-**DPhe-Arg-Trp**-Gly-Lys-Pro-Val -NH_2
MT-II	Ac-Nle-c[Asp-His-**DPhe-Arg-Trp**-Lys]-NH_2
hAGRP (87-132)	CVRLHESCLGQQVPCCDPCATCYC**RFF**NAFCYCRKLGTAMNPCSRT

FIG. 1

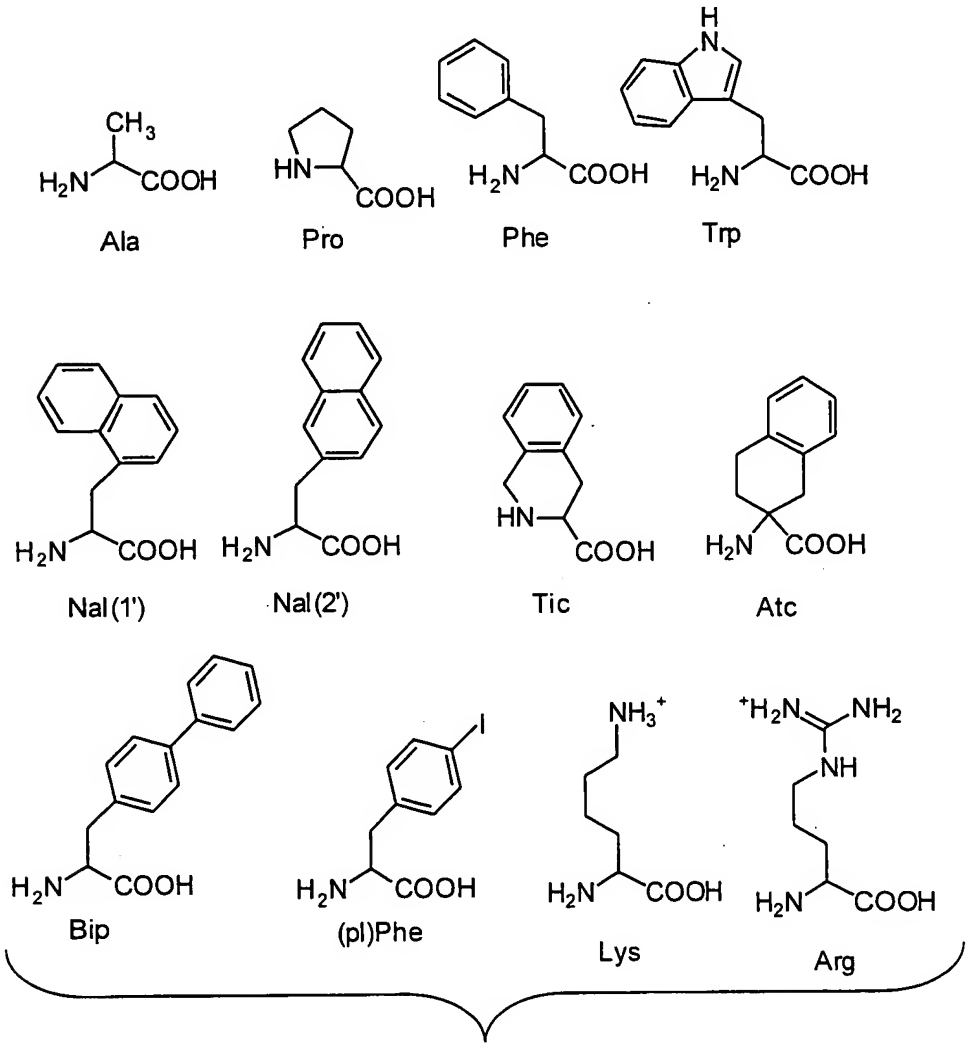

FIG. 2

DESCRIPTION

NOVEL MELANOCORTIN RECEPTOR TEMPLATES, PEPTIDES, AND USE THEREOF

Government Support

This invention was made with government support under a grant awarded from the National Institutes of Health under grant numbers DK57080 and DK64250. The government has certain rights in the invention.

Field of the Invention

The present invention relates to novel chimeric peptides and templates containing a combination of antagonist and agonist endogenous ligand residues. In particular, the present invention relates to novel chimeric peptides and templates thereof based upon melanocortin agonist peptides and agouti related protein (AGRP) antagonist peptide, and their use as drugs to treat various diseases and conditions.

Background of the Invention

Today, about two-thirds of U.S. adults are overweight or obese, according to the Centers for Disease Control and Prevention. Obesity is harmful to physical health as well as an established risk factor for a number of potentially life-threatening diseases such as atherosclerosis, hypertension, diabetes, stroke, pulmonary embolism, and cancer. Moreover, obesity can wreak havoc on an individual's mental health and can affect a person's ability to interact socially with others.

Accompanying the devastating medical consequences of this problem is the severe financial burden placed on the health care system in the United States. The estimated economic cost of obesity and its associated illnesses from medical expenses and loss of income are reported to be in excess of $68 billion per year. Because of the impact of obesity on individuals and society, much effort has been expended to find ways

to treat obesity, but little success has been achieved in the long-term treatment and/or prevention of obesity.

Pro-opiomelanocortin (POMC) derived peptides are known to affect food intake. Several lines of evidence support the notion that the G-protein coupled receptors (GPCRs) of the melanocortin receptor (MCR) family, several of which are expressed in the brain, are the targets of POMC derived peptides involved in the control of food intake and metabolism.

Five distinct MCRs have thus far been identified, and these are expressed in different tissues. MC1R was initially characterized by dominant gain of function mutations at the Extension locus, affecting coat color by controlling phaeomelanin to eumelanin conversion through control of tyrosinase. MC1R is mainly expressed in melanocytes. MC2R is expressed in the adrenal gland and represents the ACTH receptor. MC3R is expressed in the brain, gut, and placenta and may be involved in the control of food intake and thermogenesis. MC4R is uniquely expressed in the brain, and laboratory observations suggest that it is also involved in the control of food intake. See Kask A, *et al.*, "Selective antagonist for the melanocortin-4 receptor (HS014) increases food intake in free-feeding rats," *Biochem. Biophys. Res. Commun.*, 245:90-93 (1998)). MC5R is expressed in many tissues, including white fat, placenta and exocrine glands. MC5R knockout mice reveal reduced sebaceous gland lipid production (Chen *et al.*, "Exocrine gland dysfunction in MC5-R-deficient mice: evidence for coordinated regulation of exocrine gland function by melanocortin peptides," *Cell*, 91:789-798 (1997)).

Evidence for the involvement of MCRs in obesity includes: a) the agouti (A^{vy}) mouse which ectopically expresses an antagonist of the MC1R, MC3R and MC4R is obese, indicating that blocking the action of these three MCRs can lead to hyperphagia and metabolic disorders; b) MC4R knockout mice (Huszar, D. *et al.*, "Targeted disruption of the melanocortin-4 receptor results in obesity in mice," *Cell*, 88:131-141 (1997)) recapitulate the phenotype of the agouti mouse — these mice are obese; c) the cyclic heptapeptide MT-II (a non-selective MC1R, MC3R, MC4R, and MC5R agonist) injected intracerebroventricularly (ICV) in rodents, reduces food intake in several animal feeding models (NPY, ob/ob, agouti, fasted) while ICV injected SHU-9119 (MC3R and MC4R

antagonist; MC1R and MC5R agonist) reverses this effect and can induce hyperphagia; iv) chronic intraperitoneal treatment of Zucker fatty rats with an NDP-MSH derivative (HP228) has been reported to activate MC1R, MC3R, MC4R, and MC5R and to attenuate food intake and body weight gain over a 12-week period (Corcos, I. *et al.*, "HP228 is a potent agonist of melanocortin receptor-4 and significantly attenuates obesity and diabetes in Zucker fatty rats," Society for Neuroscience abstracts, 23:673 (1997)).

A specific single MCR that may be targeted for the control of obesity has not yet been identified, although evidence has been presented that MC4R signaling is important in mediating feed behavior (Giraudo, S.Q. *et al.*, "Feeding effects of hypothalamic injection of melanocortin-4 receptor ligands," *Brain Research*, 80:302-306 (1998)) and MC3R signaling may decrease food intake and participate in the regulation of energy homeostasis (obesity).

Agouti-related protein (AGRP) is a 132 (human) amino acid peptide putatively containing five disulfide bridges, and antagonizes the central brain melanocortin receptors (MC3R and MC4R) (Ollmann, M.M. *et al.*, "Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein," *Science*, 278:135-138 (1997); and Yang, Y.K. *et al.*, "Characterization of Agouti-related protein binding to melanocortin receptors," *Mol. Endo.*, 13:148-155 (1999)). Agouti (ASP) is a homologue of AGRP and was first identified as an endogenous G-protein coupled receptor (GPCR) antagonist. Both of these proteins are the only known naturally occurring antagonists of GPCRs reported to date, making them a unique family of peptides.

Previous structure-activity studies of the agouti peptide identified the importance of the three amino acid motif Arg-Phe-Phe that is conserved in both agouti and AGRP (see, for example, Kiefer, L. *et al.*, "Mutations in the carboxyl terminus of the agouti protein decrease agouti inhibition of ligand binding to the melanocortin receptors," *Biochemistry*, 36:2084-90 (1997)). These studies suggest that the conserved Arg-Phe-Phe motif found in both agouti and AGRP may be important for the antagonistic and molecular recognition properties of these two molecules at the melanocortin receptors.

All endogenous melanocortin agonists contain the putative amino acid sequence (His)/Phe-Arg-Trp, postulated to be important for melanocortin receptor molecular

recognition and stimulation. Further extrapolation of the homology between the antagonist Arg-Phe-Phe motif and the endogenous melanocortin agonist conserved residues Phe-Arg-Trp, implies that the antagonist residues may be mimicking the agonist Phe-Arg-Trp interactions with the melanocortin receptors, as supported by Tota, M.R., *et al.*, "Molecular interaction of Agouti protein and Agouti-related protein with human melanocortin receptors," *Biochemistry*, 38:897-904 (1999) and Haskell-Luevano, C., *et al.*, "The agouti-related protein decapeptide (Yc[CRFFNAFC]Y) possesses agonist activity at the murine melanocortin-1 receptor," *Peptides*, 21:683-689 (2000).

Fragments of the agouti protein have been reported to be MC1R agonists (Yang, Y.K., *et al.*, "Functional properties of an agouti signaling protein variant and characteristics of its cognate radioligand," *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 281:R1877-1886 (2001)). Various melanocortin agonist peptides (*i.e.*, Ac-His-DPhe-Arg-Trp-NH_2 and Ac-His-Phe-Arg-Trp-NH_2) have been reported to possess nM and μM potencies, respectively, at the mouse melanocortin receptors, and that the tripeptide Ac-Phe-Arg-Trp-NH_2 possesses μM agonist activity at the mMC1R (Haskell-Luevano, C., *et al.*, "Characterization of melanocortin NDP-MSH agonist peptide fragments at the mouse central and peripheral melanocortin receptors," *J. Med. Chem*, 44:2247-2252 (2001)). Further studies have shown that the Ac-His-Phe-Arg-Trp-NH_2 is the minimal fragment of melanocortin agonists required to produce a physiological response (μM) in the classic frog and lizard skin bioassay Hruby, V.J., *et al.*, "alpha-Melanotropin: the minimal active sequence in the frog skin bioassay," *J. Med. Chem.*, 30:2126-2130 (1987); and Castrucci, A.M.L., *et al.*, "Alpha-melanotropin: the minimal active sequence in the lizard skin bioassay," *Gen. Comp. Endocrinol.*, 73:157-163 (1989).

In view of the need to better understand the biology of obesity and its relationship with MCRs, novel agents, methods, and compositions for treating or preventing obesity need to be identified and developed.

Brief Summary of the Invention

The subject invention provides novel chimeric peptides based upon melanocortin agonist peptides and agouti related protein (AGRP) and methods for preparing such

peptides. The chimeric peptides of the present invention are multifunctional and demonstrate specific bioactivity at melanocortin receptors.

In one embodiment of the subject invention, amino acids His/DPhe-Arg-Trp in melanocortin agonist peptides are replaced by the AGRP Arg-Phe-Phe resides to provide a potent multifunctional chimeric peptide that is active at melanocortin receptors. In another embodiment, chimeric peptides are provided in which AGRP Arg-Phe-Phe resides are substituted with the His/DPhe-Arg-Trp amino acids of melanocortin agonists peptides. In a related embodiment, the AGRP Arg-Phe-Phe domain of the chimeric peptides described herein can include natural and/or unnatural amino acids substituted within this domain. In a preferred embodiment, the endogenous disulfide bridge between cysteine amino acids may be substituted by asparagine and diaminopropionic acid side chains of AGRP resulting in the formation of a lactam bridge. All of these embodiments present multifunction chimeric peptides that are highly potent agonists and/or antagonists of melanocortin receptors.

Brief Description of the Drawings

Figure 1 is a summary of the amino acid sequences of certain melanocortin agonists and AGRP antagonist.

Figure 2 are illustrations of amino acids and their abbreviations as described herein.

Brief Summary of the Sequences

The synthesized peptides in accordance with the present invention are based either on an AGRP(109-118) template containing melanocortin based amino acid residues, on a melanocortin agonist template containing hAGRP(111-113) Arg-Phe-Phe amino acids, or on any of the templates or peptides discussed above containing a lactam bridge as opposed to a disulfide link. For purposes of experimentation and comparison, the peptides of SEQ ID NO: 1 (Tyr-c[Cys-Arg-Phe-DPhe-Asn-Ala-Phe-Cys]-Tyr); SEQ ID NO:2 (Tyr-c[Asp-Ala-Ala-Ala-Asn-Ala-Phe-Dpr]-Tyr); SEQ ID NO:12 (Ac-Ser-Tyr-Ser-Nle-Glu-His-Ala-Ala-Ala-Gly-Lys-Pro-Val); and SEQ ID NO:19 (Ac-Nle-c[Asp-

His-Ala-Ala-Ala-Lys]) were synthesized. Peptides of the present invention include the following (from amino to carboxy terminal):

Tyr-c[Asp-Arg-Phe-Phe-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:3);

Tyr-c[Asp-Trp-Arg-Phe-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:4);

Tyr-c[Asp-Trp-Arg-DPhe-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:5);

Tyr-c[Asp-Phe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:6);

Tyr-c[Asp-DPhe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:7);

Tyr-c[Asp-His-Arg-Phe-Phe-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:8);

Tyr-c[Asp-His-Phe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:9);

Tyr-c[Asp-His-DPhe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:10);

Ac-Ser-Tyr-Ser-Nle-Tyr-c[Asp-Arg-Phe-Phe-Asn-Ala-Phe-Dpr]-Tyr-Lys-Pro-Val (SEQ ID NO:11);

Ac-Ser-Tyr-Ser-Nle-Glu-Phe-Phe-Arg-Gly-Lys-Pro-Val (SEQ ID NO:13)

Ac-Ser-Tyr-Ser-Nle-Glu-His-Phe-Phe-Arg-Gly-Lys-Pro-Val (SEQ ID NO:14);

Ac-Ser-Tyr-Ser-Nle-Glu-His-Arg-Phe-Phe-Gly-Lys-Pro-Val (SEQ ID NO:15);

Ac-Ser-Tyr-Ser-Nle-Glu-His-DArg-Phe-Phe-Gly-Lys-Pro-Val (SEQ ID NO:16);

Ac-Ser-Tyr-Ser-Nle-Glu-His-Arg-DPhe-Phe-Gly-Lys-Pro-Val (SEQ ID NO:17);

Ac-Ser-Tyr-Ser-Nle-Glu-His-Arg-Phe-DPhe-Gly-Lys-Pro-Val (SEQ ID NO:18);

Ac-Nle-c[Asp-His-Arg-Phe-Phe-Lys] (SEQ ID NO:20);

Ac-Nle-c[Asp-His-DArg-Phe-Phe-Lys] (SEQ ID NO:21);

Ac-Nle-c[Asp-His-Arg-DPhe-Phe-Lys] (SEQ ID NO:22);

Ac-Nle-c[Asp-His-Arg-Phe-DPhe-Lys] (SEQ ID NO:23);

Tyr-c[Asp-Ala-DPhe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:24);

Tyr-c[Asp-His-Ala-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:25);

Tyr-c[Asp-His-DPhe-Ala-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:26);

Tyr-c[Asp-His-DPhe-Arg-Ala-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:27);

Tyr-c[Asp-Pro-DPhe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:28);

Tyr-c[Asp-Phe-DPhe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:29);

Tyr-c[Asp-(rac)Atc-DPhe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:30);

Tyr-c[Asp-His-Pro-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:31);

Tyr-c[Asp-His-(pI)DPhe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:32);
Tyr-c[Asp-His-DNal(2')-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:33);
Tyr-c[Asp-His-DNal(1')-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:34);
Tyr-c[Asp-His-DBip-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:35);
Tyr-c[Asp-His-DPhe-Pro-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:36);
Tyr-c[Asp-His-DPhe-Lys-Trp-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:37);
Tyr-c[Asp-His-DPhe-Arg-Pro-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:38);
Tyr-c[Asp-His-DPhe-Arg-Nal(2')-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:39);
Tyr-c[Asp-His-DPhe-Arg-DNal(2')-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:40);
Tyr-c[Asp-His-DPhe-Arg-Bip-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:41);
Tyr-c[Asp-His-DPhe-Arg-Tic-Asn-Ala-Phe-Dpr]-Tyr (SEQ ID NO:42); and
Tyr-c[Asp-His-DPhe-Arg-Trp-Asn-Ala-DPhe-Dpr]-Tyr (SEQ ID NO:43).

Detailed Disclosure of the Invention

The present invention pertains to novel chimeric multifunctional peptides that are biologically active at melanocortin receptors. The peptides of the present invention are based on the identification of AGRP and melanocortin agonist domains involved in binding to melanocortin receptors. Thus, the invention provides peptides with molecular structures that duplicate or mimic the binding domains of either AGRP or a melanocortin agonist.

Accordingly, the chimeric peptides of the present invention have either an AGRP template or an MCR agonist template. Preferably, the peptides containing the AGRP peptide template have melanocortin agonist-based bioactive determinant sequences that have been substituted for the corresponding, analogous AGRP template sequences. Alternatively, the peptides containing the MCR agonist template have AGRP-based bioactive sequences which have been substituted for the corresponding melanocortin agonist template sequences. In related embodiments, the AGRP-based bioactive sequences are substituted with natural and/or unnatural amino acids. In a preferred embodiment, the peptides of the present invention can have a lactam bridge replacing the disulfide link.

Definitions

The term "patient," describes an animal, including mammals, to whom treatment with the compositions according to the present invention is provided. Mammalian species which benefit from the disclosed methods of treatment include, and are not limited to, apes, chimpanzees, orangutans, humans, monkeys; domesticated animals (*e.g.*, pets) such as dogs, cats, guinea pigs, hamsters, rabbits, rats, mice, and ferrets; and domesticated farm animals such as cows, horses, swine, sheep.

As used herein, the term "peptide," is defined as an amino acid sequence from three amino acids to about 700 amino acids in length.

The term "AGRP/MCR agonist peptides" refers to the peptides having the amino acid sequence of any of SEQ ID NOS:4-7 and 9-10, together with all related peptides described herein. The AGRP/MCR agonist peptides may or may not have amino terminal methionines, depending on the manner in which they are prepared.

The term "NDP-MSH/AGRP peptides" refers to the peptides having the amino acid sequence of any of SEQ ID NOS:12-18, together with all related peptides described herein. The NDP-MSH/AGRP peptides may or may not have amino terminal methionines, depending on the manner in which they are prepared.

The term "MTII/AGRP peptides" refers to the peptides having the amino acid sequence of any of SEQ ID NOS:20-23, together with all related peptides described herein. The MTII/AGRP peptides may or may not have amino terminal methionines, depending on the manner in which they are prepared.

Related peptides includes allelic variants; fragments; derivatives; substitution, deletion, and insertion variants; fusion polypeptides; and orthologs; and each amino acid of each such related peptide may be either natural or unatural of the "D" (natural) or "L" (unnatural) configuration which corresponds to the stereochemical designation "S" and "R," respectively, as defined in the RS system of Cahn *et al.*, (*Pure Applied Chemistry*, 45:11-30 (1974), and references cited therein). Such related peptides may be mature peptides, *i.e.*, lacking a signal peptide.

As used herein, the terms "AGRP/MCR agonist peptide variants," "NDP-MSH/AGRP peptide variants," or "MTII/AGRP peptide variants" refer to either AGRP/MCR agonist peptides, NDP-MSH/AGRP peptides, or MTII/AGRP peptides, respectively, whose amino acid sequences contain one ore more amino acid sequence substitutions, deletions, and/or additions as compared to the AGRP/MCR agonist peptide, NDP-MSH/AGRP peptide, or MTII/AGRP peptide amino acid sequences set forth in SEQ ID NOS:4-7, 9-10, 12-18, and 20-23. Such peptide variants containing amino acids of the natural L-configuration can be prepared from the corresponding nucleic acid molecule variants, which have a sequence that varies accordingly from the sequences encoding the peptides as set forth in SEQ ID NOS:4-7, 9-10, 12-18, and 20-23. Alternatively, such variants containing amino acids of the D-configuration (unnatural form) can be prepared synthetically using standard methods described herein (see also *Biochem. J.*, 219:345-373 (1984)).

The terms "AGRP/MCR agonist peptide derivatives," "NDP-MSH/AGRP peptide derivatives," or "MTII/AGRP peptide derivatives," as used herein, refer to peptides, variants or fragments thereof, that have been chemically modified, as for example, by addition of one or more water soluble polymers, N-linked or O-linked carbohydrates, sugars, phosphates, and/or other such molecules, where the molecule or molecules are not naturally attached to the peptides as set forth in SEQ ID NOS:4-7, 9-10, 12-18, and 20-23. Derivatives further include deletion of one or more chemical groups naturally attached to any of the peptides as set forth in SEQ ID NOS:4-7, 9-10, 12-18, and 20-23.

As used herein, the terms "AGRP/MCR agonist nucleic acid molecule," "NDP-MSH/AGRP nucleic acid molecule," or "MTII/AGRP nucleic acid molecule," when used to describe a nucleic acid molecule refer to a nucleic acid molecule or fragment thereof that encodes any of the peptides as set forth in SEQ ID NOS:4-7, 9-10, 12-18, and 20-23, and any fragments, derivatives, substitution, deletion, and insertion variants, fusion peptides, fusion polypeptides, and orthologs thereof.

The term "biologically active," as used herein refers to peptides that generate a functional (agonist and/or antagonist) pharmacological response at the melanocortin receptors.

For each amino acid, an additional conservative substitution includes the "homolog" of that amino acid, where the "homolog" is an amino acid with a methylene group (CH_2) inserted into the side chain at the beta position of that side chain. Examples of such homologs include, without limitation, homophenylalanine, homoarginine, homoserine, and the like.

The term "ortholog" refers to either AGRP/MCR agonist peptides, NDP-MSH/AGRP peptides, or MTII/AGRP peptides that correspond to AGRP/MCR agonist peptides, NDP-MSH/AGRP peptides, or MTII/AGRP, respectively, obtained from a species other than that from which a peptide of any of SEQ ID NOS: 4-7, 9-10, 12-18, and 20-23 was obtained.

In general, unless otherwise specified, the abbreviations used for the designation of amino acids and the protective groups used therefore are based on recommendations of the IUPAC-IUB Commission of Biochemical Nomenclature (*Biochemistry*, 11:1726-1732 (1972)). The nomenclature used to define compounds of the invention is that specified by IUPAC, published in *European Journal of Biochemistry*, 138:9-37 (1984). With regard to certain amino acids disclosed herein, their structures and abbreviations are provided in Figure 2.

Therapeutic Compositions and Administration

Therapeutic compositions of AGRP/MCR agonist peptides, NDP-MSH/AGRP peptides, or MTII/AGRP peptides are within the scope of the present invention. Such compositions may comprise a therapeutically effective amount of the peptide or fragments, variants, or derivatives in admixture with a pharmaceutically acceptable carrier. Optionally, the peptide may be formulated in an acid-salt form. The carrier material may be water for injection, preferably supplemented with other materials common in solutions for administration to mammals such as, for example, alumina, lecithin, d-α-tocopherol, polyethyleneglycol, surfactants, serum proteins such as human serum albumin, phosphates, glycine, sorbic acid, and potassium sorbate.

Typically, a AGRP/MCR agonist peptide, NDP-MSH/AGRP peptide, or MTII/AGRP peptide therapeutic compound will be administered in the form of a

composition comprising a purified peptide, fragment, variant, or derivative, optionally in its salt form, in conjunction with one or more physiologically acceptable carriers, excipients, or diluents.

Pharmaceutically acceptable salts of for the peptides of the present invention include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycolate, hemisulfate, hydrochloride, hydrobromide, hydroiodide, lactate, maleate, malonate, methanesulfonate, nicotinate, nitrate, oxalate, pectinate, phosphate, salicylate, succinate, sulfate, tartrate, thiocyanate, and other such pharmaceutically acceptable salts.

Neutral buffered saline or saline mixed with serum albumin are exemplary appropriate carriers. Preferably, the product is formulated as a lyophilizate using appropriate excipients (e.g., sucrose). Other standard carriers, diluents, and excipients may be included as desired. Other exemplary compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor.

Any AGRP/MCR agonist peptide composition, NDP-MSH/AGRP peptide composition, or MTII/AGRP peptide composition can be administered parenterally. Alternatively, such compositions may be administered intravenously or subcutaneously. When systemically administered, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such pharmaceutically acceptable protein solutions, with due regard to pH, isotonicity, stability and the like, is within the skill of the art.

Therapeutic formulations of either AGRP/MCR agonist peptide compositions, NDP-MSH/AGRP peptide compositions, or MTII/AGRP peptide compositions useful for practicing the present invention may be prepared for storage by mixing the selected composition having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company (1990)) in the form of a lyophilized cake or an aqueous solution. Acceptable carriers, excipients or stabilizers are nontoxic to

recipients and are preferably inert at the dosages and concentrations employed, and include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, pluronics or polyethylene glycol (PEG).

An effective amount of the peptide composition(s) of the present invention to be employed therapeutically will depend, for example, upon the therapeutic objectives such as the indication for which the AGRP/MCR agonist peptide is being used, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage may range from about 0.01 mg/kg to up to 1000 mg/kg or more, depending on the factors mentioned above. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the peptide) over time, or as a continuous infusion via implantation device or catheter.

The peptide compositions of the subject invention to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using these methods may be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration ordinarily will be stored in lyophilized form or in solution.

Therapeutic compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of administration of the composition is in accordance with known methods, *i.e.*, oral, injection or infusion by intravenous, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, or intralesional routes, intranasal, or by sustained release systems or implantation device which may optionally involve the use of a catheter. Where desired, the compositions may be administered continuously by infusion, bolus injection or by implantation device.

Alternatively or additionally, the composition may be administered locally via implantation into the selected area using a membrane, sponge, or other appropriate material onto which a peptide of the subject invention has been absorbed.

Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of AGRP/MCR agonist peptide, NDP-MSH/AGRP peptide, or MTII/AGRP peptide may be performed directly through the device via bolus, or via continuous administration, or via catheter using continuous infusion.

According to the present invention, an AGRP/MCR agonist peptide, NDP-MSH/AGRP peptide, or MTII/AGRP peptide may be administered in a sustained release formulation or preparation. Suitable examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, *i.e.*, films, or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al*, *Biopolymers*, 22:547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J. Biomed. Mater. Res.*, 15:167-277 .(1981) and Langer, *Chem. Tech.*, 12:98-105 (1982)), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the art (*i.e.*, Eppstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949).

The AGRP/MCR agonist peptides, NDP-MSH/AGRP peptides, or MTII/AGRP peptides, fragments, variants, and derivatives thereof, may be employed alone, together, or in combination with other pharmaceutical compositions. The peptides, fragments, variants, and derivatives of the subject invention may be used in combination with

cytokines, hormones, growth factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

Methods used for membrane encapsulation of cells are familiar to the skilled artisan, and preparation of encapsulated cells and their implantation in patients may be accomplished without undue experimentation. See, *i.e.*, U.S. Patent. Nos. 4,892,538; 5,011,472; and 5,106,627. A system for encapsulating living cells is described in PCT WO 91/10425 (Aebischer *et al.*). Techniques for formulating a variety of other sustained or controlled delivery means, such as liposome carriers, bio-erodible particles or beads, are also known to those in the art, and are described, for example, in U.S. Patent No. 5,653,975 (Baetge *et al.*, CytoTherapeutics, Inc.). The cells, with or without encapsulation, may be implanted into suitable body tissues or organs of the patient.

As discussed above, it may be desirable to treat isolated cell populations such as, for example, brain cells and/or neurons with one or more peptides, variants, derivatives and/or fragments of the subject invention. This can be accomplished by exposing the isolated cells to the AGRP/ASP peptide, variant, derivative, or fragment directly, where it is in a form that is permeable to the cell membrane.

The following examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

Abbreviations

The abbreviation "Boc" as used herein refers to *tert*-butyloxycarbonyl.

The abbreviation "DCM" as used herein refers to dichloromethane.

The abbreviation "Dde" as used herein refers to (1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl).

The abbreviation "DIPEA" as used herein refers to diisopropylethylamine.

The abbreviation "DMF" as used herein refers to dimethylformamide.

The abbreviation "DMSO" as used herein refers to dimethyl sulphoxide.

The abbreviation "EtOAc" as used herein refers to MeOH/ethyl acetate.

The abbreviation "Fmoc" as used herein refers to 9-fluorenylmethyloxycarbonyl.

The abbreviation "HOBt" as used herein refers to *N*-hydroxy-benzotriazole.

The abbreviation "MBHA" as used herein refers to methylbenzydryl-amine.

The abbreviation "PyBOP" as used herein refers to benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate.

The abbreviation "SPPS" as used herein refers to solid-phase peptide synthesis.

The abbreviation "tBu" as used herein refers to a *tert*-butyl group.

The abbreviation "TFA" as used herein refers to trifluoroacetic acid.

The abbreviation "TRH" as used herein refers to thyrotropin-releasing hormone.

The following examples illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1—Synthesis of Disulfide Crosslinked or Cyclized Peptides

As understood by the skilled artisan, disulfide cross-linked or cyclized peptides can be synthesized using standard Fmoc methodology as described in Carpino, L. A., and Han, G. Y., "The 9-Fluorenylmethyoxycarbonsy Amino-Protecting Group," *J. Org. Chem.*, 37:3404-3409 (1972); and Chang, C., and Meienhofer, J., "Solid-phase peptide synthesis using mild base cleavage of N alpha-fluorenylmethyloxycarbonylamino acids, exemplified by a synthesis of dihydrosomatostatin," *Int. J. Pept. Protein Res.*, 11:246-249 (1978). Standard Fmoc methodology can be performed on an automated or semi-automated synthesizer (Advance ChemTech 440MOS or LabTech, Louisville, KY). The amino acids Fmoc-Ser(tBu), Fmoc-Tyr(tBu), Fmoc-Nle, Fmoc-Glu(OtBu), Fmoc-His(Trt), Fmoc-Arg(Pbf), Fmoc-DPhe, Fmoc-Trp(Boc), Fmoc-Gly, Fmoc-Lys(Boc), Fmoc-Pro, Fmoc-Val, and Fmoc-Phe are all commercially available. All reagents were ACS grade or better.

Peptides of the present invention were assembled on commercially available rink-amide-MBHA resin (0.40 meq/g substitution). The synthesis was performed using a 40 well Teflon reaction block with a course Teflon frit. Approximately 200 mg resin (0.08 mmole) was added to each reaction block well. The resin was allowed to swell for 2 hrs in dimethylformamide (DMF) and deprotected using 25% piperidine in DMF for 5 min

followed by a 20 min 25% piperidine incubation at 500 rpms. A "Kaiser test," as described in Kaiser, E. *et al.*, "Color Test for Detection of Free Terminal Amino Groups in the Solid-Phase Synthesis of Peptides," *Anal. Biochem.*, 34:595-598 (1970), was applied to the resin and yielded positive results. A positive Kaiser test indicates free amine groups on the resin.

The growing peptide chain was added to the amide-resin using the following general amino acid cycle: 500 µL DMF is added to each reaction well to "wet the frit," 3-fold excess amino acid starting from the C-terminus is added (500 µL of 0.5M amino acid solution containing 0.5M HOBt in DMF), followed by the addition of 500µL 0.5M DIC in DMF and the reaction well volume is brought up to 3mL using DMF. The coupling reaction is mixed for 1hr at 500 rpms, followed by emptying of the reaction block by positive nitrogen gas pressure. A second coupling reaction is performed by the addition of 500 µL DMF to each reaction vessel, followed by the addition of 500 µL of the respective amino acid (3-fold excess), 500 µL 0.5M HBTU, 400 µL 1M DIEA, the reaction well volume is brought up to 3 mL with DMF, and mixed at 500 rpm for 1 hr. After the second coupling cycle, the reaction block is emptied and the resin-Nα-protected peptide is washed with DMF (4.5 mL 5 times). Nα-Fmoc deprotection is performed by the addition of 4 mL 25% piperidine in DMF and mixed for 5 min at 500 rpms followed by a 20 min deprotection at 500 rpms. The reaction well is washed with 4.5 mL DMF and the next coupling cycle is performed as described above.

Deprotection of the amino acid side chains and cleavage of the amide-peptide from the resin was performed by incubating the peptide-resin with 3mL cleavage cocktail (95% TFA, 2.5% water, 2.5% triisopropylsilane) for 3 hrs at 500 rpms. The cleavage product was emptied from the reaction block into a cleavage block containing 7 mL collection vials under nitrogen gas pressure. The resin was washed with 1.5 mL cleavage cocktail for 5 min and 500 rpms and added to the previous cleavage solution. The peptides were transferred to pre-weighted 50mL conical tubes and precipitated with cold (4°) anhydrous ethyl ether (up to 50 mL). The flocculent peptide was pelleted by centrifugation (Sorval Super T21 high speed centrifuge using the swinging bucket rotor) at 2000 rpm for 3 min, the ether was decanted off, and the peptide was washed one time

with cold anhydrous ethyl ether and pelleted. The crude peptide was dried *in vacuo* 48 hrs. The crude peptide yields ranged from 60% to 90% of the theoretical yields. A 7 to 15 mg sample of crude peptide was purified by RPHPLC using a Shimadzu chromatography system with a photodiode array detector and a semi-preparative RP-HPLC C_{18} bonded silica column (Vydac 218TP1010, 1.0 x 25 cm) and lyophilized. The purified peptide was >95% pure as determined by analytical RP-HPLC and had the correct molecular mass.

Disulfide bridge cyclization of the peptides synthesized above was performed in solution according to known methods, such as those described in Haskell-Luevano, C. *et al.*, "The agouti-related protein decapeptide (Yc[CRFFNAFC]Y) possesses agonist activity at the murine melanocortin-1 receptor," *Peptides*, 21:683-689 (2000); and Haskell-Luevano, C. *et al.*, "Design, synthesis, biology, and conformations of bicyclic alpha-melanotropin analogues," *J. Med. Chem.*, 38:1736-1750 (1995).

The crude linear peptide (synthesized in Example 1 above) is dissolved in 20 mL of water and 3 mL of methanol. An oxidizing solution consisting of 200 mL 0.01M potassium ferricyanide, 10 mL saturated ammonium acetate, 20 mL acetonitrile, 10 mL water, was adjusted to pH=8.5 with a few drops of concentrated ammonium hydroxide. The peptide solution was taken up in a 50 mL syringe and transferred to the oxidizing solution via a syringe pump at a rate of 1.5 mL/ h. When the transfer was complete, the pH was adjusted to 4.5 with glacial acetic acid. Amberlite resin (IRA-68 HCl form) was added to the mixture and left to mix for 45 min. The Amberlite resin was filtered off, with the solution containing the peptide concentrated, lyophilized, and purified by RP-HPLC.

Example 2—Synthesis of Peptides Containing Cyclic Lactam Bridge

In accordance with the present invention, peptides containing cyclic lactam bridges can be prepared using standard Boc methodology as described in Merrifield, R. B., "Solid Phase Synthesis. II. The Syntheis of Bradykinin," *J. Am. Chem. Soc.*, 86:304-305 (1964); and Stewart, J. M., and Young, J. D., Solid Phase Peptide Synthesis, 2nd ed., Pierce Chemical Co., Rockford, Illinois (1964) on an automated synthesizer (Advanced

ChemTech 440MOS, Louisville, KY). The amino acids Boc-Tyr(2ClBzl), Boc-diaminopropionic acid [Dpr(Fmoc)], Boc-Asp(OFm), Boc-Arg(Tos), Boc-Phe, Boc-His(Bom), Boc-DPhe, Boc-Trp(CHO), Boc-Asn, and Boc-Ala are commercially available. The peptides were assembled on commercially available pMBHA resin (0.28 meq/g substitution). All reagents were ACS grade or better.

The synthesis was performed using a commercially available 40 well Teflon reaction block with a course Teflon frit. Approximately 200 mg resin (0.08 mmole) was added to each reaction block well. With reaction volume limitations, each peptide can be synthesized in two separate reaction wells. The resin was allowed to swell for 2 hrs in 5 mL dimethylformamide (DMF) and deprotected using 4 mL 50% trifluroacetic acid (TFA), 2% anisole in dichloromethane (DCM) for 3 min followed by a 20 min incubation at 500 rpms and washed with DCM (4.5 mL, 2 min, 500 rpms 3 times). The peptide-resin salt was neutralized by the addition of 4 mL 10% diisopropylethylamine (DIEA) in DCM (3 min, 500 rpms, 2 times) followed by a DCM wash (4.5 mL, 2 min, 500 rpms 4 times). Free amino acid groups were identified on the resin using a Kaiser test.

The growing peptide chain was added to the amide-resin using the general amino acid cycle as follows: 500 µL DMF is added to each reaction well to "wet the frit," 3-fold excess amino acid starting from the C-terminus is added [400 µM of 0.5M solution in 0.5M N-hydroxybenzotriazole (HOBt) in DMF] followed by the addition of 400 µL 0.5M N,N'-diisopropylcarbodiimide (DIC) in DMF and the reaction well volume is brought up to 3mL using DMF. The coupling reaction is mixed for 1hr at 500 rpms, followed by emptying of the reaction block by positive nitrogen gas pressure. A second coupling reaction is performed by the addition of 500 µL DMF to each reaction vessel, followed by the addition of 400 µL of the respective amino acid (3-fold excess), 400 µL 0.5M O-benzotriazolyl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU), 300 µL 1M DIEA, the reaction well volume is brought up to 3 mL with DMF, and mixed at 500 rpm for 1 hr. After the second coupling cycle, the reaction block is emptied and the resin-Nα-protected peptide is washed with DCM (4.5 mL 4 times). Nα-Boc deprotection is performed by the addition of 4 mL 50% TFA, 2%anisole in DCM and mixed for 5 min at 500 rpms followed by a 20 min deprotection at 20 min. The reaction

well is washed with 4.5 mL DCM (4 times), neutralized with 10% DIEA (3 min, 500 rpms, 2 times) followed by a DCM wash (4.5 mL, 2 min, 500 rpms 4 times), and the next coupling cycle is performed as described above.

The Fmoc and OFm protecting groups are removed from Dpr and Asp, respectively by treatment with 4.5 mL 25% piperidine in DMF (20 min at 500 rpm) with a positive Kaiser test results. The lactam bridge between the Asp and Dpr amino acids is formed using 5-fold excess benziotriazolyloxy-tris-(dimethylamino) phosphonium hexafluorophosphate (BOP) and 6-fold excess DIEA as coupling agents and mixing at 500 rpms. The lactam bridges were formed (negative Kaiser test) after approximately 3 days at room temperature. Deprotection of the remaining amino acid side chains and cleavage of the amide-peptide from the resin was performed by incubation the peptide-resin with anhydrous hydrogen fluoride (HF, 5 mL, 0°C, 1hr) and 5% m-cresol, 5% thioanisole as scavengers.

After the reaction is complete and the HF has been distilled off, the peptide is ether precipitated (50 mL x 1) and washed with 50 mL cold (4°) anhydrous ethyl ether. The peptide is filtered off using a course frit glass filter and dissolved in glacial acetic acid, frozen and lyophilized. The crude peptide yields ranged from 60% to 90% of the theoretical yields. A 40 mg sample of crude peptide was purified by RP-HPLC using a Shimadzu chromatography system with a photodiode array detector and a semi-preparative reversed phase high performance liquid chromatography (RP-HPLC) C_{18} bonded silica column (Vydac 218TP1010, 1.0 x 25 cm) and lyophilized. The purified peptide was >95% pure as determined by analytical RP-HPLC and had the correct molecular mass.

Example 3—Assays

For cell culture and transfection, HEK-293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum and seeded 1 day prior to transfection at 1 to 2 x10^6 cell/100-mm dish. Melanocortin receptor DNA in the $pCDNA_3$ expression vector (20 μg) were transfected using the calcium phosphate method. Stable

receptor populations were generated using G418 selection (1mg/mL) for subsequent bioassay analysis.

Functional Bioassay

In the functional bioassay studies, HEK-293 cells stably expressing the mouse MC1, MC3, MC4 and MC5 receptors were transfected with 4μg CRE/β-galactosidase reporter gene as previously described in Haskell-Luevano, C. *et al.*, "Characterization of melanocortin NDP-MSH agonist peptide fragments at the mouse central and peripheral melanocortin receptors," *J. Med. Chem.*, 44:2247-2252 (2001); Haskell-Luevano, C. *et al.*, "Structure activity studies of the melanocortin-4 receptor by in vitro mutagenesis: identification of agouti-related protein (AGRP), melanocortin agonist and synthetic peptide antagonist interaction determinants," *Biochemistry*, 40:6164-6179 (2001); and Chen, W. *et al.*, "A colorimetric assay for measuring activation of Gs- and Gq-coupled signaling pathways," *Anal. Biochem.*, 226:349-354 (1995)). 5,000 to 15,000 post transfection cells were plated into 96 well Primera plates (Falcon) and incubated overnight. Forty-eight hours post-transfection the cells were stimulated with 100μL peptide (10^{-4} - 10^{-12} M) or forskolin (10^{-4} M) control in assay medium (DMEM containing 0.1 mg/mL BSA and 0.1 mM isobutylmethylxanthine) for 6 hrs. The assay media was aspirated and 50μL of lysis buffer (250 mM Tris-HCl pH=8.0 and 0.1% Triton X-100) was added. The plates were stored at –80° overnight.

The plates containing the cell lysates were thawed the following day. Aliquots of 10 μL were taken from each well and transferred to another 96-well plate for relative protein determination. To the cell lysate plates, 40 μL phosphate-buffered saline with 0.5% BSA was added to each well. Subsequently, 150 μL substrate buffer (60 mM sodium phosphate, 1 mM $MgCl_2$, 10 mM KCl, 5 mM β-mercaptoethanol, 200 mg/100mL ONPG) was added to each well and the plates were incubated at 37°.

The sample absorbance, OD_{405}, was measured using a 96 well plate reader (Molecular Devices). The relative protein was determined by adding 200 μL 1:5 dilution Bio Rad G250 protein dye:water to the 10 μL cell lysate sample taken previously, and the OD_{595} was measured on a 96 well plate reader (Molecular Devices). Data points were

normalized both to the relative protein content and non-receptor dependent forskolin stimulation. The antagonistic properties of these compounds were evaluated by the ability of these ligands to competitively displace the MTII agonist (Bachem) in a dose-dependent manner, at up to 10μM concentrations. The pA_2 values were generated using the Schild analysis method described in Schild, H.O., "pA, A New Scale for the Measurement of Drug Antagonism," *Brit. J. Pharmacol.*, 2:189-206 (1947).

Binding Assays

In the binding assays, ^{125}I-NDP-MSH was prepared using a modified chloramine-T method as previously described by Yang, *et al.*, "Characterization of Agouti-related protein binding to melanocortin receptors," *Mol. Endo.*, 13:148-155 (1999). Using 50 mM sodium phosphate buffer pH 7.4 as the reaction buffer, ^{125}I-Na (0.5 mCi, Amersham Life Sciences, Inc., Arlington Heights, IL) was added to 20 mg of NDP-MSH (Bachem, Torrance, CA) in 5 mL buffer. To initiate the reaction, 10 mL of a 2.4 mg/ml solution of chloramine T (Sigma Chemical Co., St. Louis, MO) was added for 15 seconds with gentle agitation. This reaction was terminated by the addition of 50 mL of a 4.8 mg/ml solution of sodium metabisulfite (Sigma Chemical Co.) for 20 seconds with gentle agitation.

The reaction mixture was then diluted with 200 mL 10% bovine serum albumin and the resultant mixture layered on a Bio-Gel P2 (Bio-Rad Labs, Hercules, CA) column (1.0 x 30 cm Econocolumn, Bio-Rad Labs) for separation by size exclusion chromatography using 50 mM sodium phosphate buffer, pH 7.4 as column eluant. Fifteen drop fractions (ca 500 mL) were collected into glass tubes containing 500 mL of 1% BSA. Each fraction was then counted on the Apex Automatic Gamma Counter (ICN Micromedic Systems Model 28023, Huntsville, AL with RIA AID software, Robert Maciel Associates, Inc., Arlington, MA) to determine peak ^{125}I incorporation fractions.

Receptor Binding Studies

One day preceding the experiment, 0.1 - 0.3 x 10^6 cells/well of HEK-293 cells (prepared and maintained as described above) were plated into Primera 24 well plates

(Falcon). The peptides (10^{-5} M) and NDP-MSH (10^{-6} to 10^{-12} M) were used to competitively displace the ^{125}I-radiolabeled NDP-MSH (100,000 cpm/well). Dose-response curves (10^{-6} to 10^{-12} M) of NDP-MSH and IC_{50} values were generated and analyzed by nonlinear least squares analysis (see Bowen, W. P., and Jerman, J. C., "Nonlinear regression using spreadsheets," *TiPS*, 16:413-417 (1995)) and the PRISM program (v3.0, GraphPad Inc.). The peptides that did not possess agonist or antagonist pharmacology in the functional assay were examined for their ability to competitively displace ^{125}I-NDP-MSH (100,000 cpm/well) at 10^{-5} M concentrations. The percent total specific binding was determined based upon the non-specific values obtained using 10^{-6} M NDP-MSH and the NDP-MSH dose response curves as controls. The standard deviation errors are derived from the average percent specific binding values from three independent experiments and using the PRISM program (v3.0, GraphPad Inc.).

Data Analysis

For data analysis, EC_{50} and pA_2 values represent the mean of duplicate experiments performed in triplet, quadruplet or more independent experiments. EC_{50} and pA_2 estimates, and their associated standard errors, were determined by fitting the data to a nonlinear least-squares analysis using the PRISM program (v3.0, GraphPad Inc.).

The peptides of the subject invention (*i.e.*, SEQ ID NOS: 3-7, 9-10, 12-18, and 20-43) were synthesized using standard procedures and purified to homogeneity using semi-preparative reversed-phased high pressure liquid chromatography as provided above in Examples 1 and 2. Table 1 summarizes the agonist EC_{50} values and antagonist pA_2 values of peptides of the present invention at the mouse melanocortin receptors, mMC1R, mMC3R, mMC4R, and mMC5R. The errors indicated in Table 1 represent the standard error of the mean determined from at least three independent experiments. The antagonist pA_2 values were determined using the Schild analysis and the agonist MTII. The value " >100,000" indicates that the compound was examined but lacked agonist or antagonist properties at up to 100 μM concentrations. Slight agonist denotes that some stimulatory response was observed at 100 μM concentrations, but not enough to determine an EC_{50} value.

TABLE 1—Pharmacological results of chimeric peptides of the present invention at the mouse melanocortin receptors

Compound	EC_{50} (nM)			
	mMC1R)	mMC3R	mMC4R	mMC5R
hAGRP (109-118)	5,120±3,040	>100,000	pA_2=6.8±0.24	>100,000
α-MSH	0.55±0.09	0.79±0.14	5.37±0.62	0.44±0.09
NDP-MSH	0.038±0.012	0.098±0.013	0.21±0.03	0.071±0.012
MTII	0.020±0.003	0.16±0.03	0.087±0.008	0.16±0.03
SEQ ID NO:1	61.3±17.9	Slight Agonist >100,000	Partial agonist pA_2=6.1±0.2	238.7±100
SEQ ID NO:2	>100,000	>100,000	>100,000	>100,000
SEQ ID NO:3	1,730±310	pA_2=5.7±0.2	pA_2=5.9±0.2	>100,000
SEQ ID NO:4	13,500±3,100	>100,000	Partial agonist 15,900±7,300	>100,000
SEQ ID NO:5	6,120±2,300	Slight Agonist	10,900±2,900	2,220±1,100
SEQ ID NO:6	19,700±4,300	Slight agonist	13,400±400	3,900±1,800
SEQ ID NO:7	4,850±1,450	Slight agonist	450±160	124±25
SEQ ID NO:8	13,300±1,700	Slight agonist	>100,000	100,000
SEQ ID NO:9	59.5±16.7	309±120	57.1±4.4	90.0±22.0
SEQ ID NO:10	0.21±0.09	0.99±0.34	0.18±0.04	0.55±0.14
SEQ ID NO:11	1,960±500	pA_2=6.2±0.3	pA_2=6.2±0.1	>100,000
SEQ ID NO:12	>100,000	>100,000	>100,000	>100,000
SEQ ID NO:13	Partial agonist 19,900±5,100	>100,000	>100,000	>100,000
SEQ ID NO:14	Partial agonist 7,220±2,200	Partial agonist 20,500±6,800	>100,000	Partial agonist 24,400±6,700
SEQ ID NO:15	59.9±8.1	480±49	930±120	327±118

Compound	EC_{50} (nM)			
	mMC1R)	mMC3R	mMC4R	mMC5R
SEQ ID NO:16	Slight agonist	>100,000	>100,000	Slight agonist
SEQ ID NO:17	7.28±0.76	6,210±990	Slight agonist	450±110
SEQ ID NO:18	3,630±320	>100,000	>100,000	Slight agonist
SEQ ID NO:19	>100,000	>100,000	>100,000	>100,000
SEQ ID NO:20	Partial agonist 38,300±8,700	>100,000	>100,000	>100,000
SEQ ID NO:21	>100,000	>100,000	>100,000	>100,000
SEQ ID NO:22	440±49	14,400±3,700	5,000±900	3,600±200
SEQ ID NO:23	2,630±830	Slight agonist	>100,000	Slight agonist
SEQ ID NO:24	7.20±2.89	29.0±7.5	0.36±0.07	0.46±0.18
SEQ ID NO:25	14000±1700	Partial agonist 30300±1400	14700±1000	690±98
SEQ ID NO:26	66.5±26.8	23800±18900	32.5±16.7	2.06±0.67
SEQ ID NO:27	350±160	13500±3700	710±170	50.1±9.6
SEQ ID NO:28	22.1±18	Partial agonist pA_2=7.2±0.2	0.63±0.20	7.16±0.22
SEQ ID NO:29	6.04±1.36	Partial agonist pA_2=7.2±0.6	0.64±0.17	3.15±1.38
SEQ ID NO:30	690±150	pA_2=6.4±0.2	115±26	440±230
SEQ ID NO:31	10300±3200	13200±3400	16700±1300	7900±4000
SEQ ID NO:32	0.30±0.05	pA_2=8.9±0.1	Partial agonist pA_2=9.4±0.4	2.33±0.96
SEQ ID NO:33	5.56±3.40	pA_2=8.3±0.2	Partial agonist pA_2=9.3±0.1	22.3±10.6
SEQ ID NO:34	9.20±0.97	320±250	0.57±0.08	3.13±1.89
SEQ ID NO:35	3.87±0.85	33.0±21.3	0.57±0.07	5.27±2.12
SEQ ID NO:36	990±290	17300±290	4300±1900	3200±2400

Compound	EC_{50} (nM)			
	mMC1R)	mMC3R	mMC4R	mMC5R
SEQ ID NO:37	680±82	30800±4200	8600±2200	800±120
SEQ ID NO:38	1000±170	39200±13200	7200±1800	700±110
SEQ ID NO:39	0.53±0.13	5.56±2.72	0.27±0.12	1.93±1.10
SEQ ID NO:40	0.20±0.05	6.50±1.87	0.67±0.25	0.95±0.40
SEQ ID NO:41	11.6±3.5	450±130	42.6±9.7	3.43±0.90
SEQ ID NO:42	21.6±10.3	3400±1200	260±99	42.7±10.0
SEQ ID NO:43	2.61±0.14	14.0±1.8	0.66±0.20	4.87±2.29

Example 4— AGRP/MCR agonist peptides

In accordance with the present invention, the hAGRP(109-118) decapeptide template was utilized to systematically replace the hAGRP(111-113) Arg-Phe-Phe antagonist amino acids with melanocortin agonist Phe-Arg-Trp residues (Table 1). For the peptide represented by SEQ ID NO:1, the Phe113 of the hAGRP(109-118) decapeptide template containing a disulfide bridge was inverted to the D-amino acid. SEQ ID NOS:2-11 replaced the disulfide bridge with a side chain lactam bridge (Asp-Dpr) to determine if the lactam bridge would result in pharmacological differences while maintaining a similar ring size as the disulfide bridge.

SEQ ID NO:2 was synthesized as a control, replacing the Arg-Phe-Phe hAGRP antagonist amino acids with Ala-Ala-Ala. The peptide of SEQ ID NO:2 lacked agonist or antagonist activity at the melanocortin receptors at up to 100μM concentrations, and was unable to competitively displace radiolabeled ^{125}I-NDP-MSH beyond a ligand 25% binding at 10μM concentrations.

SEQ ID NO:3 contains the lactam bridge instead of the disulfide bridge. Comparison of the hAGRP(109-118) to SEQ ID NO:3 resulted in nearly equipotent pharmacology, within experimental error at the mMC1R, except at the mMC4R where

the lactam bridge resulted in a 8-fold decrease in antagonist potency and μM antagonist pharmacology was detectable at the mMC3R.

Both the Phe-Arg-Trp and the Trp-Arg-Phe sequences were substituted into the hAGRP(109-118) decapeptide. Comparison of SEQ ID NOS:4 and 6, both of which contain the L-Phe configuration, resulted in μM mMC1R agonist activity while SEQ ID NO:4 lacked full agonist activity or antagonist activity at the mMC3-5Rs, and SEQ ID NO:6 resulted in μM full agonist activity at the mMC4R and mMC5R, while only possessing slight agonist activity at the mMC3R.

Previous studies of the agonist melanocortin peptides identified that inversion of chirality of Phe^7 to $DPhe^7$ of α-MSH resulted in 10-to 1000-fold increased potency. Accordingly, in SEQ ID NO:5, Trp-Arg-DPhe amino acids were substituted at the hAGRP 111-113 positions and in SEQ ID NO:7, DPhe-Arg-Trp residues were substituted at the AGRP Arg-Phe-Phe (111-113) positions to evaluate any increase in agonist activity. Consistent with previous observations for the melanocortin-based agonists, SEQ ID NOS: 5 and 7 containing the D-Phe configuration generally resulted in increased agonist potency, as compared with the corresponding L diastereoisomeric peptide, except at the mMC3R where only slight agonist activity was observed at up to 100μM concentrations.

Truncation studies of the melanocortin agonist peptides using α-MSH and NDP-MSH templates resulted in the observation that inclusion of the His^6 amino acid (α-MSH numbering) resulted in significant increased agonist potency at the melanocortin receptors. To examine the effect on enhancing agonist ligand potency of the hAGRP(109-118) template, the His^6 amino acid of the melanocortin agonist putative message sequence (His-Phe-Arg-Trp) was inserted into the lactam modified hAGRP (109-118) decapeptide template to yield the peptide of SEQ ID NO:8. In comparing SEQ ID NOS:8 and 3, insertion of the His residue into the AGRP(109-118) template resulted in a 8-fold decreased mMC1R agonist potency, but antagonist activity was absent at the mMC3 and mMC4 receptors and SEQ ID NO:8 bound to these receptors (mMC3R and mMC4R) at less than 50% specific binding.

Substitution of the His residue (His-Phe-Arg-Trp) for the His-Arg-Phe-Phe motif of SEQ ID NO:8 resulted in the generation of the peptide represented by SEQ ID NO:9. SEQ ID NO:9 possesses nM full agonist potency at all the examined melanocortin receptors.

SEQ ID NO:10, which contains the agonist His-DPhe-Arg-Trp sequence in the AGRP antagonist template, resulted in sub nM agonist potency at the mMC1R and mMC3-5Rs, within experimental error of α-MSH at these receptors, with the exception that at the mMC4R, SEQ ID NO:10 was ca 30-fold more potent than α-MSH.

The N-terminal Ac-Ser-Tyr-Ser-Nle and C-terminal Lys-Pro-Val-NH_2 amino acids of the melanocortin agonist NDP-MSH were substituted at the respective peptide termini of the lactam bridge hAGRP (109-118) decapeptide to yield the peptide of SEQ ID NO:11. SEQ ID NO:11 resulted in nearly equipotent melanocortin receptor pharmacology as SEQ ID NO:3 that lacked the N- and C-terminal agonist amino acid extension, supporting the hypothesis that the central "core" residue 109-118 region of the hAGRP antagonist determines melanocortin receptor potency and pharmacology.

In accordance with the present invention, natural and/or unnatural amino acids were substituted within the melanocortin agonist Phe-Arg-Trp residues utilized to replace the hAGRP(111-113) Arg-Phe-Phe amino acids to yield the peptides of SEQ ID NOS:24-43. SEQ ID NOS:28-30 resulted in antagonist activity at mMC3R and agonist activity at mMC4 and mMC5 receptors.

Example 5— NDP-MSH/AGRP peptides

In accordance with the present invention, the linear tridecapeptide NDP-MSH agonist template was used as a base in which DPhe-Arg-Trp amino acids were replaced with the hAGRP(111-113) Arg-Phe-Phe residues. The peptide of SEQ ID NO:12 was synthesized as a control peptide having the agonist DPhe-Arg-Trp residues replaced with Ala-Ala-Ala. SEQ ID NO:12 resulted in a complete loss of agonist activity at up to 100 μM concentrations and was unable to bind to the MC3R or MC4R more than 25%. Thus, the bioactivity of the control peptide of SEQ ID NO:12 verifies the importance of the DPhe-Arg-Trp residues for melanocortin receptor activity.

The peptide of SEQ ID NO:13 has the NDP-MSH linear tridecapeptide template substituted with hAGRP residues in the Phe-Phe-Arg orientation and deletion of the His^6 residue (α-MSH numbering). SEQ ID NO:13 resulted in a lack of agonist or antagonist activity at up to 100 μM at the MC3-5 receptors, but was a partial agonist at the MC1R.

In contrast, the peptide of SEQ ID NO:14 contains the HIS^6 amino acid and the AGRP residues in the Phe-Phe-Arg orientation. SEQ ID NO:14 resulted in partial agonist activities and no antagonist activity at the MC1R, MC3R, and MC4R with little observable binding or activity at the MC4R.

Incorporation of the hAGRP(111-113) residues into the NDP-MSH template in the Arg-Phe-Phe, resulted in the peptide of SEQ ID NO:15. Peptides of SEQ ID NO:15 have nM melanocortin receptor agonist potency.

These results, and those presented above for the hAGRP(109-118) template, demonstrate that the hAGRP(111-113) Arg-Phe-Phe antagonist residues mimic the agonist Phe-Arg-Trp amino acids in a similar topographical orientation as the linear sequences. As discussed previously, it has been well documented that inversion of chirality of the melanocortin agonist Phe^7 (α-MSH numbering) to the D configuration resulted in ligands possessing enhanced melanocortin receptor potency.

To correlate which hAGRP(112-113) Phe residue corresponds to the melanocortin agonist Phe^7 amino acid in regards to putative ligand-receptor interactions, systematic stereochemical inversion of the hAGRP (111-113) Arg-Phe-Phe residues in the peptide of SEQ ID NO:15 resulted in SEQ ID NOS:16-18. Generally, for SEQ ID NOS:16-18, stereochemical inversion of the Arg-Phe-Phe residues in the NDP-MSH template resulted in dramatic decreases in melanocortin receptor activity, with the exception of SEQ ID NO:17, as compared with SEQ ID NO:15.

SEQ ID NO:17 (NDP-MSH linear template) containing the DPhe that putatively corresponds to the hAGRP Phe^{112} residue, resulted in only an 8-fold increase in mMC1R agonist potency, a 13-fold decreased mMC3R agonist potency, conversion from a full mMC4R agonist to only a slight agonist at 100μM concentrations and equipotent mMC5R agonist potency, compared with SEQ ID NO:15.

Example 6—MTII/AGRP peptides

In accordance with the present invention, the cyclic heptapeptides MTII agonist template was used as a base in which DPhe-Arg-Trp amino acids were replaced with the hAGRP(111-113) Arg-Phe-Phe residues to synthesize the peptides of SEQ ID NOS:20-23. The peptide of SEQ ID NO:19 was synthesized with the MTII DPhe-Arg-Trp residues substituted with Ala-Ala-Ala as a control to the peptides of SEQ ID NOS:20-23.

With the peptide of SEQ ID NO:20, the DPhe-Arg-Trp amino acid sequence of cyclic MTII was substituted with the hAGRP(111-113) Arg-Phe-Phe residues. SEQ ID NO:20 resulted in a loss of full agonist activity at the MC3-5 receptors and only possessed μM partial agonist activity at the MC1R.

Stereochemical inversion of the hAGRP(111-113) Arg-Phe-Phe residues of SEQ ID NO:20 resulted in the peptide of SEQ ID NO:21, wherein DArg-Phe-Phe was incorporated into the MTII peptide template. The peptide of SEQ ID NO:21 lost the ability to generate a full agonist response at up to 100μM concentrations at the melanocortin receptors.

However, SEQ ID NO:22 (MTII cyclic template) containing the DPhe that putatively corresponds to the hAGRP Phe^{112} residue, resulted in converting the peptide with the corresponding L-Phe isomer (SEQ ID NO:20) from an mMC1R partial agonist into an nM full agonist, and a ligand possessing full μM agonist activity at the mMC3-5 receptors.

These results suggest that the antagonist hAGRP Phe^{112} may be mimicking the melanocortin agonist $DPhe^{7}$ interactions with the receptor in the cyclic hAGRP(109-118) and MTII peptide templates, in terms of enhancing general melanocortin receptor agonist potency. Although this latter speculation remains to be experimentally verified, this is the first experimental evidence suggesting that the hAGRP Phe^{112} antagonist residue may topographically correlate with the melanocortin agonist Phe^{7} amino acid in terms of putative ligand-mMC4R interactions.

The peptide of SEQ ID NO:23 contains the Arg-Phe-DPhe motif substituted for the DPhe-Arg-Trp amino acids in the MTII peptide template. SEQ ID NO:23

demonstrated slight agonist activity at 100 μM at the MC3R and MC5R, but was a μM MC1R agonist.

Example 7—Melanocortin Receptor Selective Ligands

The central MC3 and MC4 receptors expressed in the brain have been associated with the physiological role of weight and energy homeostasis through the use of knockout mice and *in vivo* feeding studies (see Fan, W. *et al*, "Role of melanocortinergic neurons in feeding and the agouti obesity syndrome," *Nature*, 385:165-168 (1997); Huszar, D. *et al.*, "Targeted disruption of the melanocortin-4 receptor results in obesity in mice," *Cell*, 88:131-141 (1997); Butler, A.A. *et al.*, "A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse," *Endocrinology*, 141:3518-21 (2000); and Chen, A.S. *et al.*, "Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass," *Nat Genet*, 26:97-102 (2000)). Due to the neuroanatomical overlap in some regions of the brain of the MC3 and MC4 receptor mRNA and the complexity of energy homeostatic pathways, melanocortin ligands selective for either of these melanocortin receptor isoforms are desirable for *in vivo* studies.

In the subject invention, the peptide of SEQ ID NO:7 (Tyr-c[Asp-DPhe-Arg-Trp-Asn-Ala-Phe-Dpr]-Tyr-NH_2) resulted in a ligand that is only a slight mMC3R agonist (is not a mMC3R antagonist, does not bind at the mMC3R greater than 25% specific binding at 10μM concentrations, but possess a 450nM agonist EC_{50} value at the mMC4R resulting in a >200-fold MC4R versus MC3R selective compound.

The peptide of SEQ ID NO:17 (Ac-Ser-Tyr-Ser-Nle-Glu-His-Arg-DPhe-Phe-Gly-Lys-Pro-Val-NH_2), resulted in a potent nM mMC1R agonist possessing high nM agonist activity at the mMC5R, μM agonist activity at the mMC3R and only slight agonist activity at the mMC4R (not an antagonist and does not bind to the mMC4R at greater than 25% specific binding at 10μM concentrations. Thus, SEQ ID NO:17 is a 850-fold MC1R versus MC3R selective, >16-fold MC4R versus MC3R selective, and 62-fold MC1R versus MC5R selective peptide.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

DECLARATION (37 C.F.R. § 1.63) AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name; and

I believe that I am the original, first, and sole inventor (if only one name is listed below), or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **Novel Melanocortin Receptor Templates, Peptides, and Use Thereof** , specification for which

☒ is attached hereto.
☐ was filed _____, Serial No. _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 and/or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Application Serial No.	Country	Filing Date	Priority Claimed

I hereby claim priority benefits under Title 35, United States Code §119 of any provisional application(s) for patent listed below:

Application Serial No.	Filing Date	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, §120 and/or §365 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (Patented, Pending, Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following persons registered to practice before the Patent and Trademark Office as my attorneys with full power of substitution and revocation to prosecute this application and all divisions and continuations thereof and to transact all business in the Patent and Trademark Office connected therewith: John M. Sanders, Reg. No. 30,126; David R. Saliwanchik, Reg. No. 31,794; Jeff Lloyd, Reg. No. 35,589; Doran R. Pace, Reg. No. 38,261; Jay M. Sanders, Reg. No. 39,355; Jean Kyle, Reg. No. 36,987; James S. Parker, Reg. No. 40,119; Frank C. Eisenschenk, Reg. No. 45,332; Glenn P. Ladwig, Reg. No. 46,853; Margaret Efron, Reg. No. 47,545; and Gwendolyn L. Daniels, Reg. No. 51,594.

I request that all correspondence be sent to:

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Name of First or Sole Inventor: Carrie Haskell-Luevano

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Date: _____

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Signature of First or Sole Inventor